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THE EFFECT OF CHLORINATION ON SELECTED ORGANIC CHEMICALS

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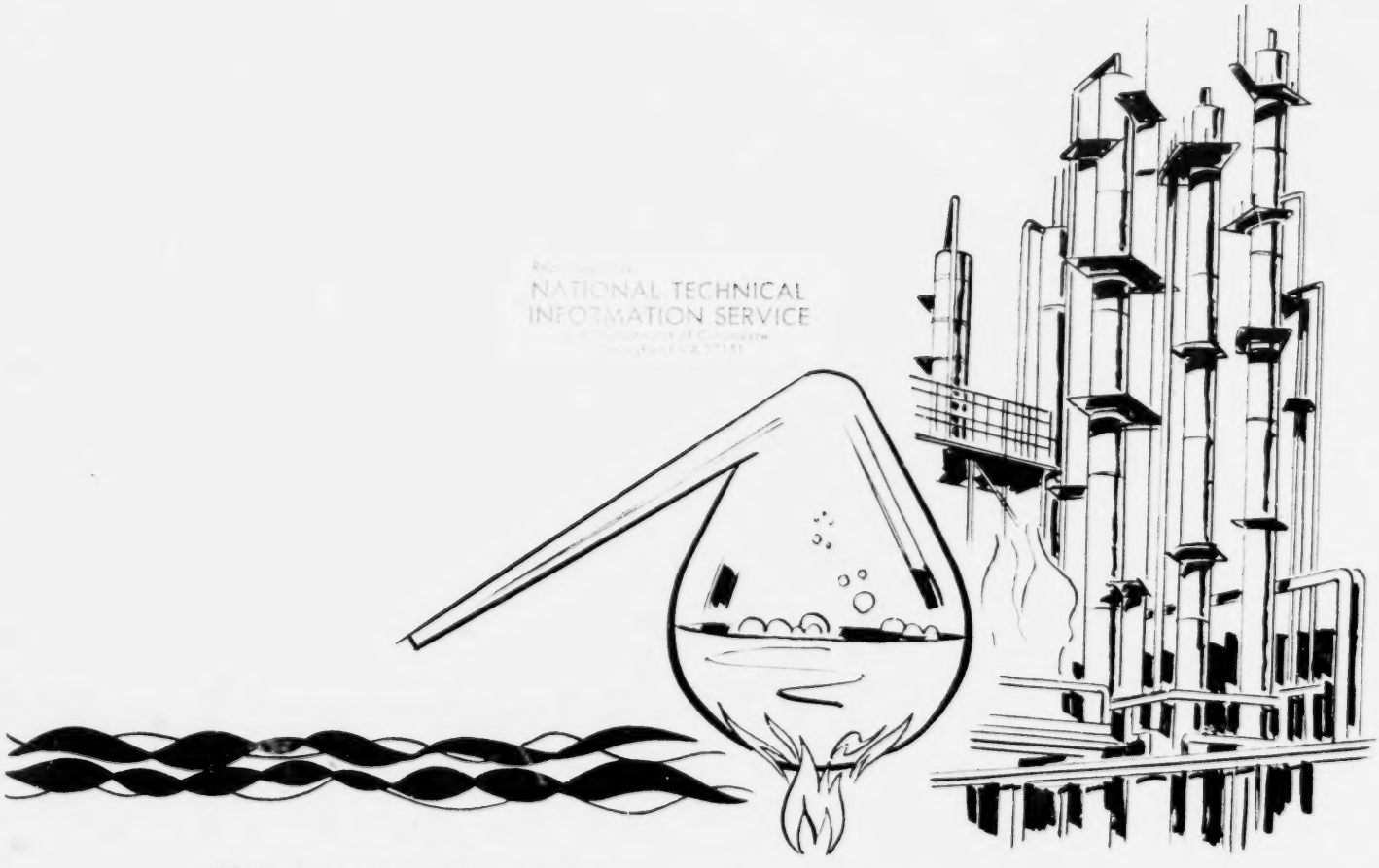

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EFFECT OF CHLORINATION ON SELECTED ORGANIC CHEMICALS,

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~~It was found that~~ certain of the test compounds formed persistent degradation products during treatment. Five of the initial compounds reacted readily with chlorine, under conditions commonly employed in effluent chlorination.

Five of the chlorination products were further studied in respirometer experiments to evaluate their persistence in mixed microbial systems. Their toxicity to fish was determined using the static bioassay procedure.

In the ~~final phase of the study~~, a series of bench scale, continuous flow ecosystems were established for the evaluation of longer term effects of three of the chlorination products. Several varieties of organisms, representing different levels in the food chain, were studied. (Lowry - Texas)

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Mark V. Lowry
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THE EFFECT OF CHLORINATION ON SELECTED ORGANIC CHEMICALS

by

The Manufacturing Chemists Association
1825 Connecticut Avenue, North West
Washington, D.C. 20009

for the

Office of Research and Monitoring

ENVIRONMENTAL PROTECTION AGENCY

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ABSTRACT

The results of this study indicated that chlorination of effluents containing certain organic chemicals can result in the formation of stable reaction products, which may or may not contain chlorine. It was further shown that there compounds exercise a retardant influence on aquatic life.

Fourteen industrial organic chemicals were examined for their persistence through biological treatment as the initial compounds, or as degradation products. Semi-continuous activated sludge systems were employed. The ability of each of the chemicals to participate in reactions with free chlorine was then determined in a series of batch experiments.

Certain of the test compounds formed persistent degradation products during treatment. Five of the initial compounds reacted readily with chlorine, under conditions commonly employed in effluent chlorination.

Five of the chlorination products were further studied in respirometer experiments to evaluate their persistence upon exposure to a heterogeneous microbial population. Their toxicity to fish was determined using the static bioassay procedure.

Finally, a series of bench scale, continuous flow ecosystems was established for the evaluation of longer term effects of three of the chlorination products. Several varieties of organisms, representing different levels in the food chain, were studied.

This report was submitted in fulfillment of project #12020EXG under the partial sponsorship of the Water Quality Office, Environmental Protection Agency.

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SECTION I

CONCLUSIONS

Phase I: Chlorination Studies and Analytical Methods

1. Gas chromatographic analysis was successfully employed for the detection of most of the initial test compounds in aqueous solutions. The method was applied to the analysis of effluents from bench scale biological systems, and was used in monitoring the test chemicals during chlorination experiments.

2. Ultraviolet absorption (UV) spectrophotometry demonstrated applicability to the measurement of aromatic compounds in relatively pure aqueous solutions. The method was used in compound monitoring during the bioassay experiments, and provided information on the nature of the products resulting from chlorination.

3. There is evidence to indicate the formation of degradation products of several of the test compounds, during biological treatment, in acclimated systems.

4. Five of the fourteen chemicals selected for study were observed to participate in reactions with free chlorine, under conditions encountered during conventional effluent chlorination practice. The ability of these compounds to react with chlorine can be related to the structural characteristics of the chemicals.

5. Evidence indicates that chlorine reacts with these organic chemicals by both substitution and oxidation, resulting in a highly complex mixture of products. In some cases, it was possible to identify the products of reaction.

6. The nature and distribution of the products of reaction with chlorine are affected by a variety of parameters, including concentrations and contact time.

Phase II: Chlorination Product Persistence and Toxicity

7. Several of the reaction products identified in Phase I were examined in respirometer studies and found to be resistant to degradation upon exposure to a heterogeneous microbial population.

8. Toxicity to fish by these products was demonstrated in static bioassay experiments, and 96-hour TL_m values were established.

9. On the basis of the results developed in Phases I and II, it is evident that chlorination of effluents containing certain organic chemicals may result in the formation of persistent and potentially deleterious reaction products.

Phase III: Intermediate Bioassay Experiments

10. Considering the relative efforts involved, the intermediate term, flow through bioassays as conducted did not provide significantly better insight into the toxic effects of the test compounds, than did the routine four-day static bioassays. The static test appears to provide a conservative toxicity limit with minimum effort.

11. Although some qualitative judgments of compound effects on the test microorganism populations can be drawn, the data were too variable to allow quantitative statistical determinations of toxicity. Qualitative evidence was suggestive of toxic or inhibitory effects on stalked ciliates and diatoms for each of the three compounds at the highest concentration levels tested.

12. A significant increase in effort, well above that practical for a project of this nature, would be needed to provide statistically definable determinations of toxicity to microorganisms.

13. Of the two types of vascular plants tested, one exhibited an erratic growth pattern and was unsuitable as an indicator of toxicity. The second type was not erratic in growth, but no toxicity was observed. The general outward appearance of all plants was not adversely affected by any of the compounds.

14. Stable macroinvertebrate populations could not be maintained in the model ecosystems for a time period of suitable duration for compound testing.

15. On the level of experimentation performed, the energy and food web interrelationships within the experimental ecosystems could not be defined.

SECTION II

RECOMMENDATIONS

1. The practice of routine chlorination of industrial effluents should be re-examined. In cases where it has been demonstrated or suspected that organic chemicals are reacting with chlorine to produce undesirable products, an alternative disinfection method should be considered.

2. Effluents known to contain chlorine reactive materials should be monitored for those materials, and for unusual increases in chlorine demand.

3. The present study should be extended to provide further information regarding the nature and properties of the products of reaction between chlorine and industrial chemicals, which have escaped biological treatment.

4. Further studies should be undertaken to determine the effects of these products of chlorination on biological systems. These studies should include evaluations of fish toxicities.

5. The intermediate term, flow through bioassay such as employed herein, is not recommended for future investigations of compound toxicity. By comparison, the routine four-day static bioassay provides a simple method which yields a conservative determination of toxicity to a test organism, at considerable saving of effort.

SECTION III

INTRODUCTION

The chlorination of raw or treated domestic wastewaters is commonly practiced to achieve disinfection and deodorization. In many municipalities, effluent chlorination is mandated by public health codes. In recent years, this country has witnessed a trend towards combined municipal-industrial waste treatment. Additionally, an increasing number of industries have been required to provide wastewater treatment facilities, which often includes effluent chlorination. These developments have raised serious questions regarding the impact of certain industrial chemicals on wastewater treatment plant operation in general, and on chlorination in particular. It is conceivable that, under certain conditions, some organic compounds may escape treatment or be only partially degraded, such that they are available for reaction with chlorine in the contact chamber. Furthermore, the products of such a reaction could, upon discharge, exercise a deleterious effect on the receiving stream.

A typical example of such an undesirable reaction is the combination of chlorine with phenol to produce the chlorophenols. These materials are a source of obnoxious tastes and odors, even at very low concentrations (µg/l).

Moreover, studies by Ingols and Jacobs[1] have shown that the chlorophenols are more resistant to biodegration than phenol itself. Trichlorophenol was cited as being toxic to phenol-adapted microorganisms. Chambers, et.al.[2] also reported on the increased resistance of the chlorinated products of both phenol and m-cresol to biodegradation. In an extension of an earlier work, Ingols, et.al.[3] determined that the chlorophenols were more toxic to fish than phenol.

The preceeding considerations clearly indicate the need for a re-examination of effluent chlorination practice. In addition, research efforts should be directed towards identifying those chemicals which are present in wastewater effluents and which will react with chlorine. Information should also be developed on the impact of these materials on the ecology of a receiving water.

In recognition of these problems, the Manufacturing Chemists' Association, in cooperation with the Environmental Protection Agency, initiated the present study for the purpose of gathering information on the effect of chlorination on certain industrial organic compounds.

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SECTION IV

STUDY OBJECTIVES

The present study was undertaken in an attempt to develop information on the effects of chlorination on selected organic chemicals. The need to develop testing procedures and analytical methods for the characterization of these effects was judged to be of paramount importance. Specific project objectives included:

1. The examination of the influence of selected organic compounds and/or their degradation products on chlorine demand.

2. The identification of those chemicals which form stable reaction products upon contact with chlorine, under conventional conditions of chlorination.

3. The characterization of those reaction products in terms of their persistence in biological systems, or their potential to act as inhibitors or toxicants to such systems.

4. The determination of the effects of any persistent reaction products on the ecology of a simulated receiving stream.

From an organizational standpoint, the study was arranged into three distinct phases, as shown in Table 1. Each successive phase was intended to represent a logical investigative progression in the accomplishment of the objectives of the project. It was envisioned that some effort in each of the phases would proceed concurrently, since the nature of the studies necessitated the refinement or development of experimental and analytical methodologies. Consequently, subsequent descriptions of the studies are not intended to be chronologically consistent.

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TABLE 1

ORGANIZATION OF THE PROJECT

Chemicals Selected For Study	Phase I	Phase II	Phase III
Alcohols Methanol (1°) Isopropanol (2°) T-Butanol (3°) Ketones Acetone Benzene and Derivatives Benzene Toluene Ethylbenzene Benzoic Acid Phenol and Phenolics Phenol m-Cresol Hydroquinone Organic Nitrogen Compounds Aniline Dimethylamine Nitrobenzene	All compounds tested for: 1. suitability of analytical methods 2. persistence of parent or degradation product through biological treatment 3. reaction with chlorine 4. identification and characterization of products	Five compounds selected on the basis of Phase I experimental results: 1. evaluation of resistance to biodegradation 2. determination of toxicity	Three compounds selected on the basis of information gathered in Phases I and II — for evaluation of effects on a simulated ecosystem

SECTION V

GENERAL DESCRIPTION OF PHASE I

Phase I was devoted to the examination of the influence of chlorination on specific industrial chemicals, before and after biological treatment. Fourteen compounds, representing several classes of organic chemicals, were chosen for these initial investigations, as indicated in Table 1.

The selection of these compounds was made on the basis of their industrial importance either as primary products or as chemical intermediates. Each of the chemicals listed is known to appear in industrial wastewater effluents.

Preliminary efforts during the first phase of the study included the definition of chemical and physical characteristics of each of the test compounds. A listing of these properties is given in Table 2. This list was compiled in part from data given in standard texts[4]. Information on the response of each of the compounds to BOD and COD analysis was established experimentally.

The introduction of organic chemicals to a biological treatment system, e.g., the activated sludge process, may result in either of two effects: (1) complete conversion of the starting compounds to carbon dioxide, water, new microbial cells, and possibly, some intermediate compounds; or (2) incomplete degradation or no degradation, such that the starting compounds appear in the effluent. In continuous systems, some residual organic material will always remain in solution as a result of an equilibrium between the microbial cells and their liquor.

The compositions of treated effluents are highly complex, and have been characterized only in terms of general classes of materials, e.g., carbohydrates, proteins, tannins, "fulvic" and "humic" acids. The nature and distribution of specific components is known to be affected by selection of treatment parameters, as well as by the composition of the feed stock. A well functioning continuous biological system, receiving a feed stock of uniform composition, will achieve essentially complete conversion of the organic material initially present. Such a system is said to be acclimated, in contrast to a system which is unacclimated. A biological process which is subjected to intermittent or shock loads may be unacclimated to certain components associated with these loads.

At this juncture, it is useful to review the conditions under which a biological system achieves acclimatization. This may be visualized by a consideration of the generalized biological treatment process, as illustrated in Figure 1. It is noted that the growth of a microbial population (sludge) progresses through several phases upon exposure to a substrate: first, a

TABLE 2

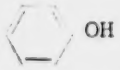
CHEMICAL AND PHYSICAL CHARACTERISTICS OF TEST COMPOUNDS

Compound	Formula	Molecular Weight	Boiling Point °C	Solubility (mg/100ml)	Density (g/ml)	Total Oxygen Demand (mg/mg)	BOD_5 (mg/mg)	A.A.* COD (mg/mg)	Reflux COD (mg/mg)
Methanol	CH_3OH	32.04	64.65	∞	0.796	1.50	1.0	1.395	1.46
Isopropanol	$CH_3-\underset{OH}{CH}-CH_3$	60.09	82.3	∞	0.785	2.39	1.45	1.79	2.34
T-Butanol	$CH_3-\overset{CH_3}{\underset{OH}{C}}-CH_3$	74.12	108.4	9,500	0.801	2.59	0	1.65	2.46
Benzene		78.11	80.1	82	0.879	3.07	0	0.13	0.82
Ethylbenzene		106.16	136.1	14	0.867	3.16	0	0.05	0.89
Toluene		92.13	110.6	47	0.867	3.13	0.86	0.13	1.58
Benzoic Acid		122.12	249	270	1.266	1.96	1.42	1.83	1.87

*Technicon AutoAnalyzer procedure.

TABLE 2
(continued)

CHEMICAL AND PHYSICAL CHARACTERISTICS OF TEST COMPOUNDS

Compound	Formula	Molecular Weight	Boiling Point °C	Solubility (mg/100ml)	Density (g/ml)	Total Oxygen Demand (mg/mg)	BOD_5 (mg/mg)	A.A.* COD (mg/mg)	Reflux COD (mg/mg)
Phenol		94.11	182	6,700	1.072	2.38	1.75	1.785	2.32
M-Cresol		108.13	202.8	2,350	1.034	2.52	1.70	1.23	2.31
Hydroquinone		110.11	286.2	5,000	1.358	1.89	0.75	1.83	1.66
Aniline		93.12	184.4	3,400	1.022	3.18	1.8	1.67	2.47
Nitrobenzene		123.11	210.9	190	1.198	3.05	0	0.19	1.39
Dimethylamine	$CH_3\underset{H}{N}CH_3$	45.08	7.4	V.S.	0.68	3.75	0	0	0
Acetone	$CH_3-\overset{\overset{O}{\|}}{C}-CH_3$	58.08	56.5	∞	0.792	2.20	1.4	1.13	1.80

*Technicon AutoAnalyzer procedure.

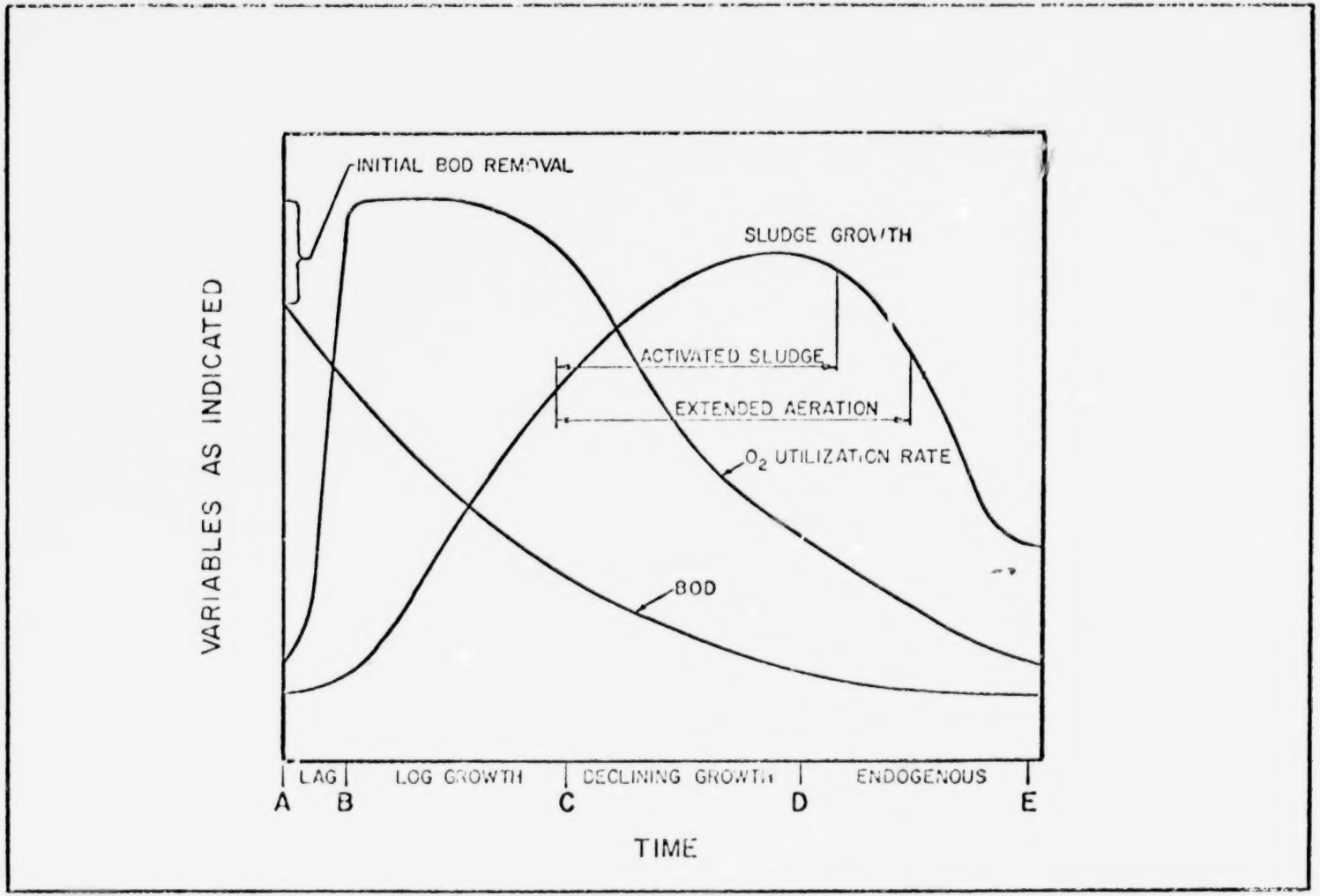


FIGURE I

SCHEMATIC REPRESENTATION OF BIOLOGICAL TREATMENT PROCESS

lag phase, characterized by adaptation of the culture from the previous environment to the present; second, a period of maximum growth under conditions where unlimited food is available; third, a period of declining growth where food availability becomes a limiting condition; and finally, an endogenous phase where, under severely limited food conditions, cells die and are, in turn, consumed such that the mass population is reduced. In heterogeneous systems, this process occurs within the biological culture for each substrate as a subset of the overall reaction. In a system which is unacclimated to a particular compound, the lag phase associated with that substrate may be prolonged, and may result in the passage of that compound through the treatment process, without any significant degradation.

In the evaluation of the effects of chlorine on organic chemicals subjected to biological treatment, two cases should be considered: (1) an investigation of the possible reactions of chlorine with the parent compounds which would persist through unacclimated systems; and (2) the determination of the probability of chlorine reactions with degradation products of the starting compounds, which would appear in the effluents from acclimated biological systems.

The case of chlorine reaction with possible degradation products was examined first. Requisite to the evaluation of potential effects of chlorination is the establishment of the existence of significant degradation products which uniquely result from biological treatment of any of the test compounds. In certain cases, the presence or absence of degradation products can be inferred from a consideration of the nature of the reactants and their known degradation characteristics. A simple substrate, such as methanol, should undergo virtually direct conversion to carbon dioxide, water, and cellular material. For more complex organic molecules, degradation may be accomplished by a series of reactions, involving intermediate forms.

A series of experimental tests can be applied for the determination of intermediate product production. Ideally, the detection of the intermediate product itself would be the most direct approach. However, no single analytical technique is universally applicable, and the selection of a method must necessarily be based on some information about the material being detected. In the absence of direct experimental evidence, indirect procedures are often employed in the determination of the presence of degradation products. Monitoring of the effluent for the disappearance of the parent compound (by chromatographic or spectrophotometric procedures, for example) and for a decrease in oxygen demand may provide some useful information. If the rate of compound disappearance closely parallels the rate of COD removal associated with that compound, it may be generally asserted that no significant degradation product buildup has occurred. On the other

hand, if a net residual COD is observed, relative to a control system, intermediate product formation may be taking place.

The lack of adherence to stoichiometry for oxygen utilization may provide additional evidence for the production of intermediates. If the complete disappearance of a parent compound is accompanied by substantially less than theoretical oxygen consumption required to produce CO_2, water, and cells, there is likelihood for the existence of degradation products.

Experience has shown that no single technique, among those described above, is conclusive in demonstrating the presence or absence of significant intermediate products which are uniquely associated with the biological degradation of a specific compound. Indeed, even the application of all these procedures only leads to a "probable" result, due to the inherent inadequacies of these experimental and analytical tests. The following is a partial list of some of the conditions which would tend to diminish the significance of the experimental results:

1. partial or complete volatilization of test compound or intermediate product during biological treatment;

2. variable recovery (sensitivity) of the COD test to parent compound or product;

3. direct chemical degradation (auto-oxidation, photochemical reaction, etc.) of compound(s).

The nature of the biological treatment process itself is not conducive to the maintenance of true steady-state conditions. Transient changes in microbial population dynamics may yield erratic results. Background levels of residual byproduct material from cellular lysis (endogenous respiration) may obscure the presence of specific intermediate compounds.

Perhaps even more pertinent to the evaluation of the probability of chlorine reactions with degradation products is the fact that the nature and distribution of degradation products may be controlled by the selection of treatment parameters. As an example, ammonia (NH_3) may be regarded as a byproduct of organic nitrogen degradation. However, by appropriate adjustment of loading conditions (food-to-microorganism ratio), the conversion of ammonia to oxidized nitrogen forms ($NO_2 - NO_3$) may be encouraged. Furthermore, it is known that ammonia will participate in reactions with chlorine, leading to the formation of chloramines, but nitrate undergoes no reaction with chlorine.

In summary, it may be concluded that:

1. present methods for establishing the existence of intermediate products resulting from the biochemical degradation of specific organic chemicals lack general applicability and are of questionable validity;

2. the nature and distribution of degradation products may be drastically altered by adjustment of treatment parameters;

3. due to recognized experimental limitations, the more practical case for investigation is the ability of chlorine to react with specific organic chemicals, which have escaped biological treatment.

In accordance with the considerations detailed above, the major effort of Phase I was devoted to the examination of the ability of chlorine to react with each of the initial test compounds. Test conditions were selected to simulate those that would be anticipated for full scale treatment plants: dilute solutions of organic compounds, pH ranges near neutrality, conventional applied chlorine dosages and contact times. It should also be pointed out that all applied chlorine was in the form of free chlorine, since ammonia-free solutions were used. The principal criterion applied in the determination of chlorine-organic chemical reactions was that of the chlorine demand, as derived from residual chlorine analysis. Chromatographic techniques provided the means for monitoring the concentrations of test compounds during the chlorination experiments and, in some instances, facilitated the identification of reaction products. Supplemental information was also gained by the use of ultraviolet absorption spectrophotometry.

SECTION VI

EXPERIMENTAL METHODS – PHASE I

Analytical Procedures

In view of the need for monitoring of the test compounds throughout the experimental program, a substantial portion of the effort in Phase I was devoted to the development of analytical techniques. It was recognized that any method worthy of consideration should have general applicability to the range of selected organic chemicals and their derivatives, be capable of selective identification of specific components in complex systems, and be suitable for quantitative analysis at low (mg/l) concentrations.

Liquid-gas chromatography was initially chosen for these investigations. The instrument used was a Perkin-Elmer Model 881 chromatograph with flame ionization detector and linear temperature programing. Helium was employed as the carrier gas, while hydrogen and air were supplied to the flame ionization detector. Chromatograms were recorded on a Leeds and Northrup Speedomax W recorder equipped with a Disc integrator.

Five columns were employed for chromatographic separations during these studies, as listed below:

1. 6' × 1/8" (uncoated) Poropak Q, 100/120 mesh, packed in stainless steel

2. 6' × 1/8" (uncoated) Poropak S, 150/200 mesh, packed in stainless steel

3. 6' × 1/8" 15% K20M Carbowax TPA on Chromosorb W HDMS, 80/100 mesh, packed in stainless steel

4. 12' × 1/8" 15% K20M Carbowax TPA on Chromosorb W HDMS, 60/80 mesh, packed in glass

5. 6' × 1/8" Chromosorb 103, 80/100 mesh, packed in glass

In all cases, dual column systems were utilized to facilitate baseline stability by compensating for column "bleeding" effects.

For purposes of parameter optimization and calibration, standard solutions of each of the test compounds were prepared in distilled water. Where available, chromatographic or reagent

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grade chemicals were used. Injection of samples was accomplished with a Hamilton microliter syringe of suitable size (1, 5, or 10 µl). Calibration curves were constructed by plotting the product of the attenuation factor and area (relative Disc integrator units) as a function of sample weight. Standardization was checked frequently during the experimental program to insure quantitative reproducibility.

It became evident that chromatographic techniques, employing direct aqueous injections of samples, were not applicable to the separation and quantitative detection of some of the compounds under investigation. It therefore was decided to explore the use of UV spectrophotometry as an analytical tool.

The instrument used for these studies was a Perkin-Elmer Model 202 double-beam ratio-recording spectrophotometer. Standard solutions of each of the compounds of interest were prepared in distilled water. Absorbance-wavelength recordings were obtained in the 190-350 nm region of the spectrum, using distilled water as the reference. Calibration curves were prepared by plotting absorbance as a function of concentration at each peak wavelength. Ultraviolet spectral scans for samples dissolved in a solvent other than distilled water employed the appropriate solvent in the reference cell.

Biological Degradation Studies

A series of semi-continuous activated sludge systems, each receiving one of the selected compounds, in combination with the necessary nutrients, was employed for these studies. Each system consisted of a two-liter aeration chamber containing activated sludge which had been initially obtained from a local domestic waste treatment plant. Sufficient dissolved oxygen levels and mixing were provided by diffused air fed into the bottom of each unit. Feeding was accomplished on a daily schedule, according to the following procedure: (1) the air supply was temporarily cut off and the biological solids allowed to settle; (2) a one-liter portion of supernatent was removed by siphoning and set aside for analysis; (3) the appropriate quantity of feed solution was dispensed into each unit, and the final volume was re-adjusted to two liters with tap water. In all cases, the feed consisted of 100 mg/l of each test compound, expressed as Theoretical Oxygen Demand (TOD), in combination with 100 mg/l (as COD) of a synthetic "sewage", containing a mixture of readily degradable organic compounds and inorganic nutrients. A control unit, which received 200 mg/l (as COD) of the synthetic mixture was also maintained during these studies. This synthetic media was prepared at frequent intervals in accordance with the following formula:

SYNTHETIC DOMESTIC WASTE COMPOSITION FOR A COD OF 100,000 MG/L

Ingredient	grams/liter
skim milk	48
peptone	48
gelatin	16
soluble starch	32
urea	8
disodium hydrogen phosphate	8
KCl	1.12
$CaCl_2$	1.12
$MgSO_4$	0.80
$Fe_2(SO_4)_3$	0.80
NH_4Cl	8

Following a two-week period of acclimatization, each of the systems was monitored for reduction in soluble COD and for disappearance of test substrate, where chromatographic techniques were available. Analyses were performed immediately after addition of test substrate and synthetic mixture, and at selected intervals thereafter.

In an aerated biological reactor, substrate removal may also occur by diffused air stripping of volatile components. To investigate the significance of this phenomenon for each of the test chemicals, a separate series of tests was conducted using the same apparatus as previously described. In these experiments, the biological culture was omitted, and an aqueous solution of each test chemical was aerated, using an air flow rate similar to that employed in the degradation studies. The concentration of each test compounds was monitored with time, using chromatographic or spectrophotometric procedures.

Chlorination Experiments

To determine the effect of chlorine on each of the selected compounds, two series of batch chlorination experiments were conducted. The first set of tests were designed to identify, in a qualitative sense, which of the selected chemicals were capable of reacting with chlorine, under conventional treatment conditions.

Aqueous solutions, containing approximately 10 mg/l of each compound, were prepared using distilled water and adjusted to pH 7.4, with a phosphate (K_2HPO_4–KH_2PO_4) buffer. Where possible, the concentration of test chemical was checked using chromatographic procedures. A stock chlorine solution, having an approximate concentration of 1,000 mg Cl_2/l, was prepared from a commercial grade of sodium hypochlorite. This solution was standardized frequently by iodimetric titration in accordance with the procedures described in Standard Methods[5].

Each test run consisted of four (4) 500 ml samples containing the test compound, to which varying dosages of chlorine were applied. Since a gradual loss of chlorine with time had been demonstrated in preliminary tests, a control solution containing only 10 mg/l of chlorine in phosphate-buffered distilled water was included in each experiment. The four solutions of test compound received nominal chlorine dosages of 0, 5, 10, and 20 mg/l. Mixing was accomplished on a multiple stirring ("jar test") apparatus, using 1" × 3" stainless steel paddles rotating at 80 rpm. Solution temperatures were maintained at 25°C ± 1°C.

Samples were withdrawn at 0.5, 1.0, 2.0, and 24 hours after chlorine addition and immediately analyzed for free and combined residual chlorine. The orthotolidine (OT) and orthotolidine-arsenite procedures, as described in Standard Methods,[5] were followed in these determinations. Where chromatographic calibrations were available, the samples were also analyzed for the presence of the test compound. A sufficient quantity of sodium thiosulfate was added to each sample prior to chromatographic analysis to destroy the free chlorine residual. This step was taken to preclude the possibility of any extraneous compound-chlorine reactions during exposure to the elevated temperature conditions of the chromatographic column.

Those compounds which had been observed to react with chlorine in the preliminary chlorination experiments were subjected to further examination in a series of detailed tests. The experimental procedures were essentially the same as described above. A wider range of applied chlorine dosages (up to 100 mg/l) was employed in these studies in an attempt to further elucidate the stoichiometry of these reactions. Ultraviolet absorption spectrophotometric procedures were used to provide supplemental information on parent compound disappearance and reaction product formation.

SECTION VII

RESULTS OF PHASE I

Analytical Methods

On the basis of previous experience, chromatographic techniques appeared to offer promise for the separation and quantitative detection of the compounds selected for study. It was envisioned that these procedures could also provide the means for the identification of products of biological degradation and/or chlorination.

Of the fourteen chemicals initially selected, reliable chromatographic techniques were developed for seven. These compounds were amenable to chromatographic analysis at low (mg/l) concentrations using the Poropak Q column. Peaks were well separated from the water response and observed to be symmetrical, with little or no tailing. Reasonable response times were obtained for these materials, as summarized in Table 3. Note that the relative retention times of these species are sufficiently different, so as to allow the resolution of each of the components in a complex mixture.

TABLE 3

CHROMATOGRAPHIC ANALYSIS OF TEST COMPOUNDS
RETENTION DATA

Column: 6' × 1/8" Poropak Q 100/120 Mesh (SS)

Compound	Carrier Flow (ml/min)	Column Temperature (°C)	Retention Time (min)
Methanol	35	120	2.5
Isopropanol	35	150	5.5
T-Butanol	35	150	9.5
Acetone	35	150	4.0
Benzene	35	160	11.25
Toluene	35	190	10.0
Ethylbenzene	20	225	7.0

As a result of the lack of suitable gas chromatographic methods for the analysis of certain of the test compounds, ultraviolet absorption spectrophotometric procedures were also evaluated during the Phase I studies. The results of preliminary tests confirmed the suitability of UV analysis for the determinations of several of the test compounds, including

phenol, m-cresol, nitrobenzene, aniline, and hydroquinone. Dimethylamine was found to be inactive in the UV region. The absorption characteristics of the compounds tested are summarized in Table 4. Subsequent experiments also revealed the utility of UV analysis for chlorinated product identification and monitoring. These studies will be detailed in a later section of the report.

TABLE 4

ULTRAVIOLET ABSORPTION CHARACTERISTICS OF TEST COMPOUNDS

Compound	λ_1 (nm)	λ_2 (nm)	λ_3 (nm)
Phenol	207	235	288
m-Cresol	222	271	277
Hydroquinone	193	220	288
Aniline	199	230	280
Nitrobenzene	195	214	270
4-chloro-3-methylphenol*	∿200	279	287
2,4,6-trichlorophenol*	∿214	245	312
2,4,6-trichloroaniline*	∿210	242	304
p-benzoquinone*		245	

*Studied in Phases II and III

Biological Degradation Studies

The purpose of this phase of the investigation was to identify the test compounds or their degradation byproducts which could react with chlorine under conditions that occur in municipal waste treatment. Two conditions of treatment must be identified; first the acclimated condition, where the test substrate is in the system continuously or for a sufficient period for acclimatization of the biological system to occur, and second, the unacclimated, or partially acclimated system. The unacclimated condition is the result of a non-regular discharge of a particular chemical to the treatment plant. Both conditions regularly occur in practice.

Acclimated Systems

The setup of the physical systems has previously been described. The control systems received a synthetic "sewage" with a COD strength of 200 mg/l. Each of the test streams were composed of a mixture containing 100 mg/l COD of synthetic sewage and 100 mg/l as COD (calculated basis) of the test substrate. The effluent from each test system was examined to identify the component or its degradation products in the effluent.

Except in the case where the test substrate or its known degradation products are directly measureable, a significant determination problem results. This problem can, in most cases, be surmounted by considering supplementary data on COD removal, and degradation studies on the particular compound. Employing a combination of the analytical information, it is possible to examine each of the test compounds. Table 5 summarizes the study results.

TABLE 5

SUMMARY OF BIOLOGICAL DEGRADATION STUDIES

Compound	Results
Isopropanol	Complete loss of primary substrate (gas chromatography). Identifiable intermediate acetone.
Methanol	Complete loss of primary substrate (gas chromatography). No identifiable degradation product.
m-Cresol	Biological oxidation of substrate.
Phenol	Biological oxidation of substrate.
Acetone	Complete loss of primary substrate (gas chromatography). No identifiable degradation product.
Benzene	Removal by stripping and biooxidation.
Toluene	Removal by stripping and biooxidation.
Ethylbenzene	Removal by stripping and biooxidation.
Benzoic Acid	Presumptive evidence of complete loss of primary substrate with no degradation product formation — biological oxidation.
Hydroquinone	Possible substrate persistence or degradation product formation.
Nitrobenzene	Complete loss of primary substrate (gas chromatograph). Possible formation of unidentified degradation product.
Dimethylamine	Presumptive evidence for some primary substrate survival. Ammonia is a product of biological oxidation.
T-Butanol	Partial survival of primary substrate, degradation product formation unknown.
Aniline	Possible survival of primary substrate suspected. Degradation product formation suspected.

Figure 2 presents batch study data illustrating the disappearance of isopropanol with a corresponding increase in acetone,

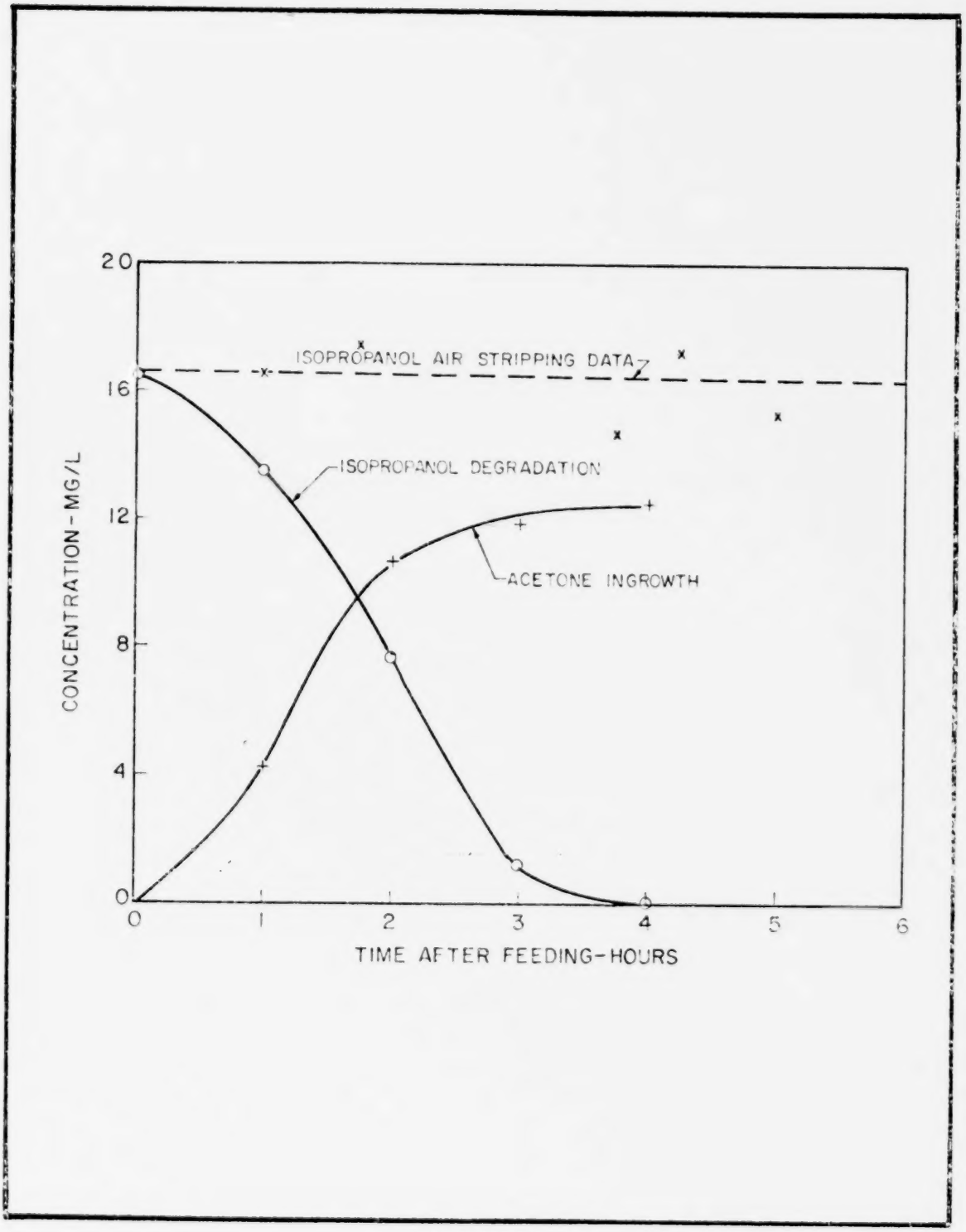


FIGURE 2

BIOLOGICAL DEGRADATION OF ISOPROPANOL

as determined by chromatographic analysis. A complete stoichiometric evaluation of the completeness of reaction was difficult because of acetone loss by stripping. No evidence of any other product formation was uncovered. Acetone itself was eventually lost from the system at longer aeration times (see below).

Methanol disappearance, as measured by gas chromatography, and COD disappearance data indicated that methanol is completely degraded without the production of an intermediate product. Comparison studies indicated that stripping is not a major factor in removal, as shown in Figure 3.

Both phenol and m-cresol could be tracked by gas chromatography and COD analysis. A typical gas chromatographic output for m-cresol is presented in Figure 4. In another experiment, the phenol concentration was reduced from 33.7 mg/l to 4.7 mg/l after one hour. An analysis of residual COD data, as shown in Table 5, indicated that no intermediate products were present in detectable quantity.

Acetone was monitored by gas chromatography and showed complete disappearance from the test units. COD data (refer to Table 6) indicated no accumulation of an additional product. Examination of the removal pathway indicated that a significant portion of the acetone was removed by stripping, as evident from Figure 4. Approximately 50% of the removal was accomplished by biological oxidation.

TABLE 6

SUMMARY OF COD DATA FROM BIOLOGICAL DEGRADATION STUDIES

Compound	Theoretical Total COD Added†	Initial COD Recoverable By Test*	Residual COD (mg/l) at 24-hr Average
Acetone	200	151	41.6
Isopropanol	200	175	50.1
m-Cresol	200	150	44.7
Methanol	200	193	49.3
Phenol	200	195	41.8
Benzene	200	104	48.5
Ethylbenzene	200	101	56.2
Toluene	200	104	45.3
Benzoic Acid	200	200	38.4
Hydroquinone	200	197	39.0
Nitrobenzene	200	106	52.4
Dimethylamine	200	100	55.9
T-Butanol	200	164	69.9
Aniline	200	152	68.5
CONTROL	200	200	56.1

†Includes 100 mg/l synthetic sewage (as COD).
*Calculated from Table 2.

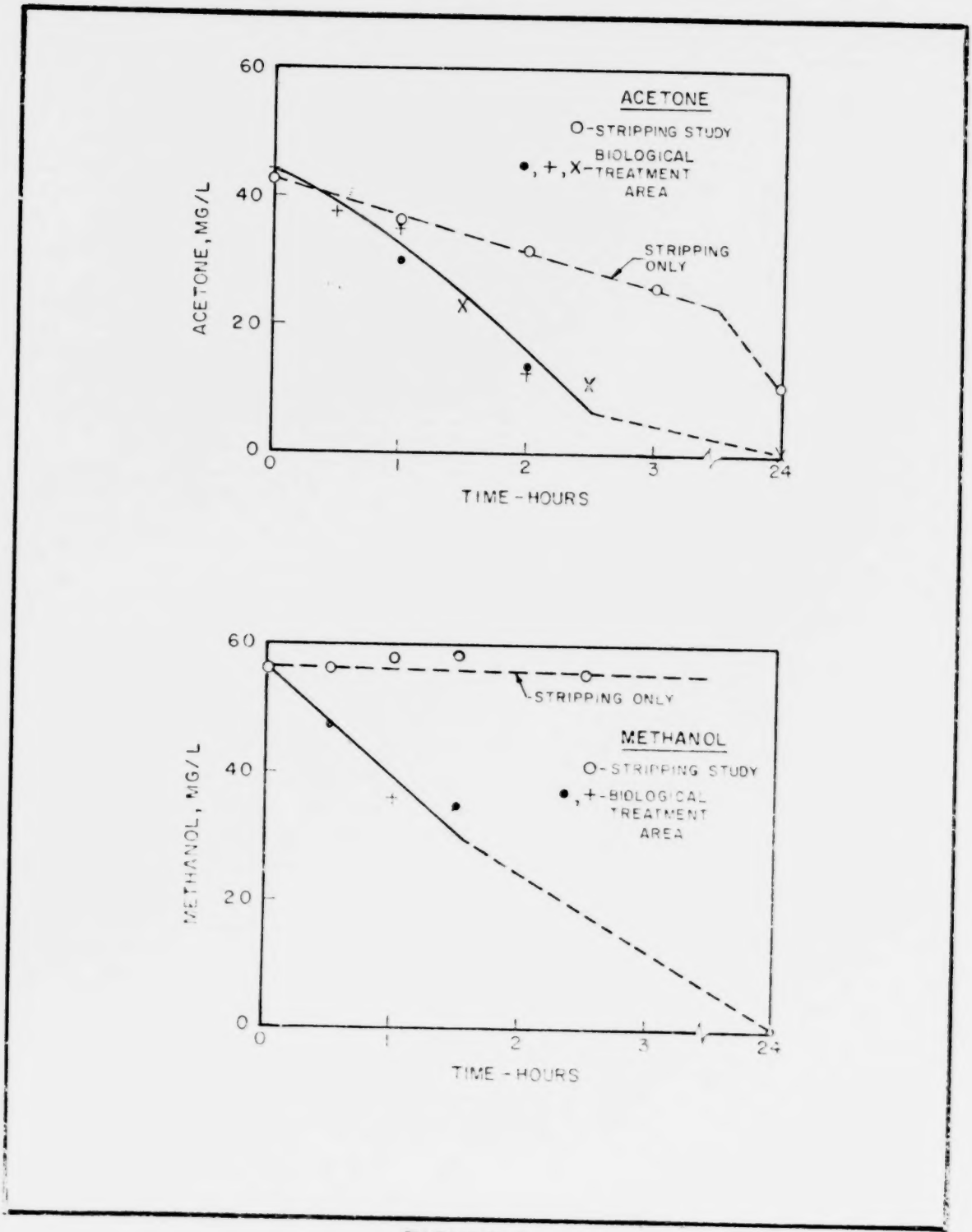


FIGURE 3

BIOLOGICAL DEGRADATION OF ACETONE AND METHANOL

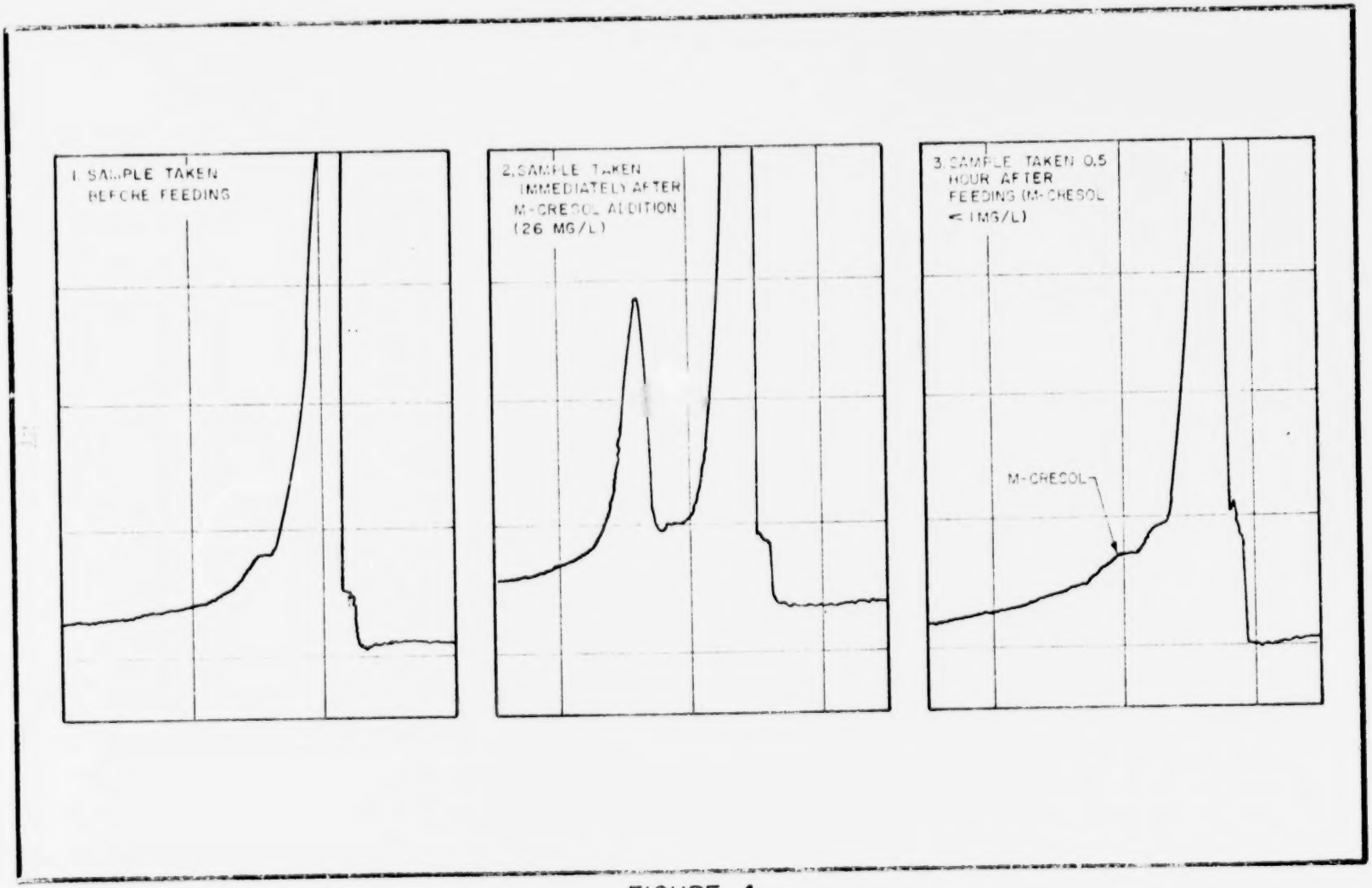


FIGURE 4

CHROMATOGRAMS ILLUSTRATING M-CRESOL BIODEGRADATION

The removals of benzene, toluene, and ethylbenzene were monitored by gas chromatography. Studies indicated that they were, to a very significant degree, removed from the system by stripping. If any biological degradation products were formed by a small portion of these materials that were actually oxidized, they were below detection limits.

A primary substrate method for evaluating benzoic acid presence was not available. COD data (refer to Table 6) indicated complete oxidation of the substrate with no apparent degradation products. For supporting evidence, a sample was studied for the regularity of oxygen use with both acclimated and unacclimated seed. The results of this study are shown in Figure 5. The regularity of both curves supports the contention that the reaction is complete, with no significant intermediates being formed. Although the evidence is not totally conclusive, there is sufficient presumptive evidence to infer the improbability of degradation product formation.

The lack of a totally satisfactory method of analysis made determination of hydroquinone in low concentrations impractical. The substrate was substantially degraded, as shown by the residual COD data in Table 6. However, indirect oxygen utilization data suggested imcomplete oxidation, or possible degradation product formation. Possible products were not identified experimentally.

Gas chromatographic studies indicated that nitrobenzene disappeared from solution during the study period. Analysis by ultraviolet absorption indicated that removal by air stripping was insignificant. COD and oxygen use data suggested the formation of a degradation product of the reaction. The product could not be identified experimentally in this study. The product concentration was in the order of 10%-15% of the original nitrobenzene as COD.

Although no satisfactory method of analysis was available for low concentrations of dimethylamine, there is presumptive evidence that some primary substrate persists for a long period during biological treatment. Ammonia was found as a degradation product of oxidation.

T-butanol degrades very slowly and is detectable even after exhaustive periods of biological oxidation. No degradation product was identified, but considering the amount of the t-butanol present, it cannot be positively concluded that a product does not exist. Typical degradation data are shown in Figure 6.

No suitable analytical method was available for low concentrations of aniline. Based on COD data, (Table 6) it may be concluded that either aniline or a degradation product remains, even after substantial time. The analytical evidence is insufficient to draw a valid conclusion. However, indirect oxygen utilization studies suggested the persistence of aniline through biological systems, particularly in the presence of mixed substrates.

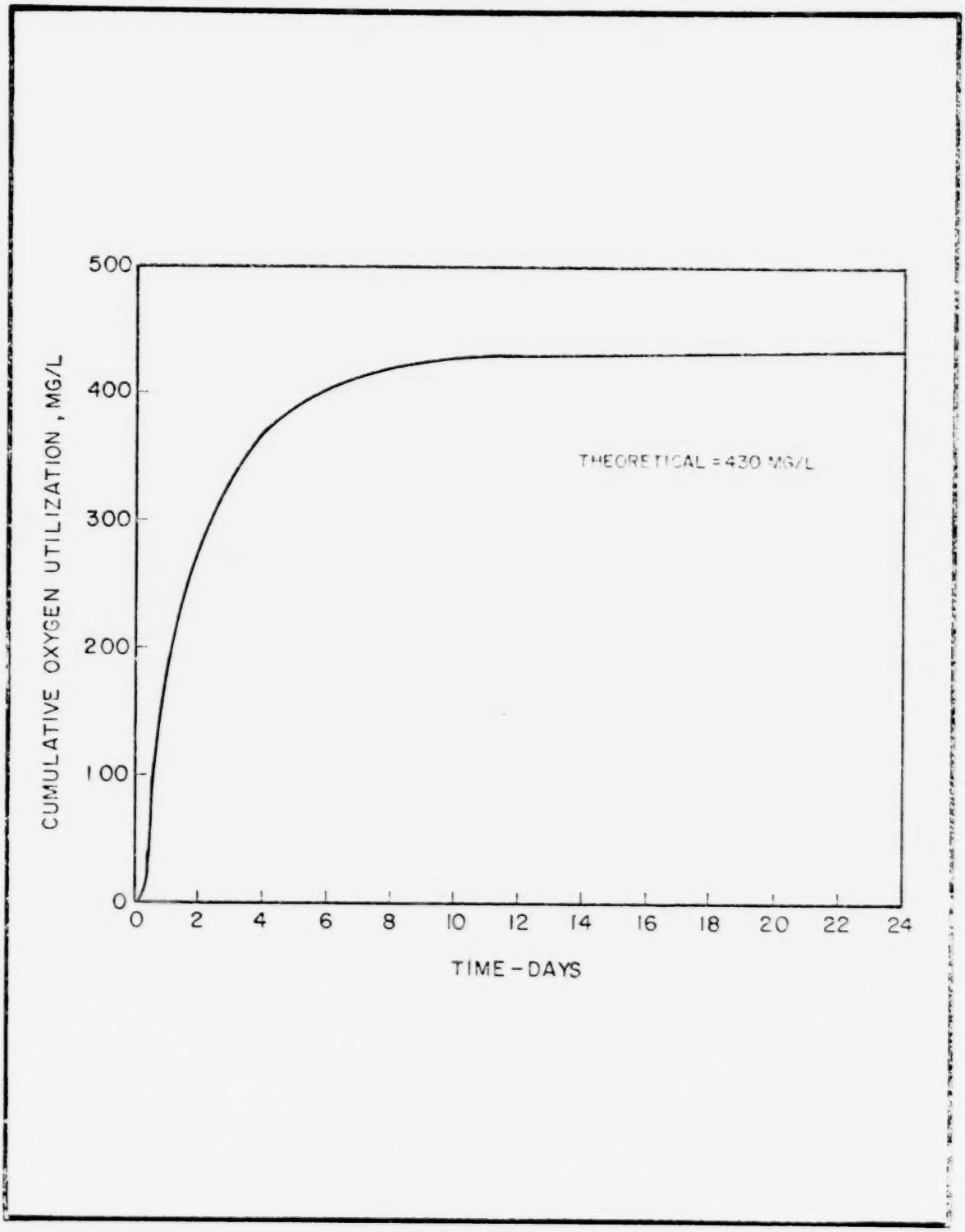
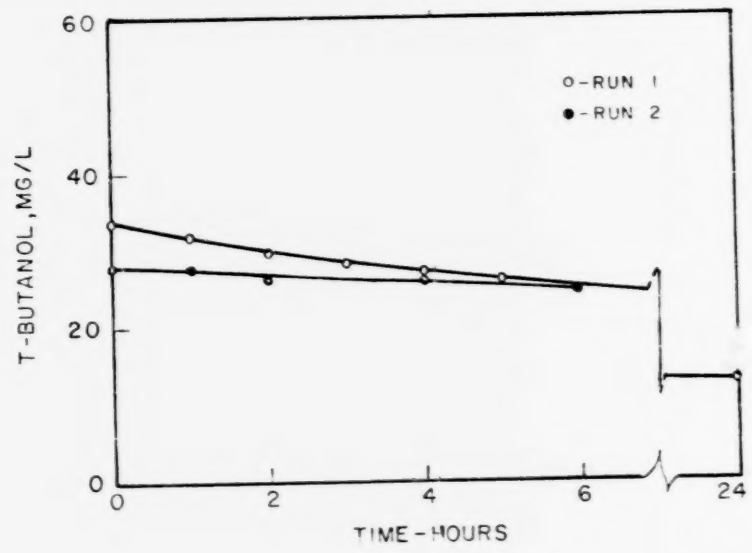
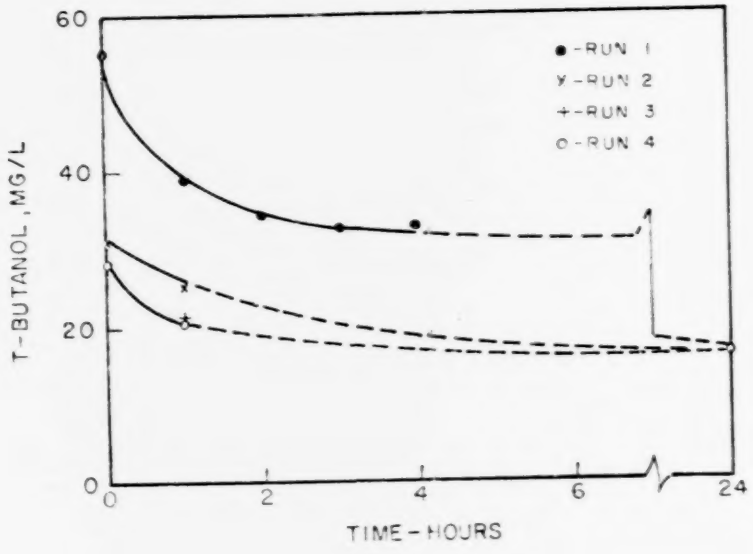


FIGURE 5

BOD PROGRESSION OF BENZOIC ACID



VOLATILIZATION OF T-BUTANOL



BIOLOGICAL TREATMENT OF T-BUTANOL

FIGURE 6

Unacclimated Systems

In an unacclimated system, the discharge may contain any of the products found in the acclimated system, as well as the original test substrate. The concentration and distribution of products will depend on the actual conditions of discharge and treatment.

Discussion of Results

The results of the biological degradation studies indicated possible degradation product formation in acclimated systems, for the following test substrates:

aniline
hydroquinone
nitrobenzene
t-butanol
isopropanol
dimethylamine

Ammonia was determined to be the product of dimethylamine degradation, and acetone was identified as an intermediate in isopropanol breakdown. Due to the limitations of the available analytical methods, degradation products of the remaining materials could not be confirmed or identified. As a consequence, it was decided to concentrate efforts on the determination of problems associated wih chlorination of the original test chemicals. This approach has direct application to "spill" situations, in which a substrate passes through an unacclimated biological system, without degradation. Such occurrences are commonplace in industrial wastewater treatment practice.

Chlorination Experiments

The first series of chlorination experiments was designed to determine which of the selected chemicals were capable of reacting with free chlorine under conditions commonly encountered in conventional treatment plants. A portion of these preliminary investigations was devoted to the evaluation of the precision of the residual chlorine test and the stability of chlorine in test solutions. A precision of 5% (relative standard deviation) was obtained from a statistical treatment of thirteen replicate chlorine residual determinations on control solutions having nominal chlorine concentrations of 10 mg/l. In accordance with this observation, a free chlorine demand of 10% of the applied dosage was the criterion used to determine any significant compound–chlorine reaction.

The results of the first series of compound chlorination experiments are given in Table 7, which lists the chlorine demand as a function of applied chlorine dosage and contact time for each of the test chemicals. The data indicate that, under the test conditions, no significant chlorine demand was exercised by the following compounds:

TABLE 7

RESULTS OF PRELIMINARY CHLORINATION EXPERIMENTS

Compound (mg/l)	Nominal Applied Chlorine (mg/l)	Chlorine Demand (OTA) At Indicated Contact Time, hours*			
		0.5	1.0	2.0	24.0
Methanol, 8	5	-0.3	-0.6	-0.1	-0.5
	10	-1.0	-0.7	-1.2	0
	20	-0.6	-1.2	-0.2	-1.0
Isopropanol, 10	5	-0.1	+0.1	-0.4	+0.3
	10	-0.6	-0.1	-0.9	+0.6
	20	-0.7	-0.8	-0.7	+0.8
T-Butanol, 10	5	-0.2	0	0	+0.4
	10	-0.2	-0.2	0	0
	20	-0.2	+0.2	-0.2	+0.6
Acetone, 10	10	0	0	-0.4	-
	20	-0.1	-0.4	-0.8	-
Acetone, 20	10	-0.7	0	-0.7	-
	20	-0.1	+0.2	+0.2	-
Benzene, 10	5	-0.1	-0.1	-0.2	-0.6
	10	0	0	-0.2	-0.3
	20	-0.2	-0.3	+0.3	-0.7
Toluene, 10	5	0	+0.1	-0.4	-0.3
	10	+0.4	+0.3	+0.3	-0.4
	20	-0.2	-0.3	-0.2	-0.4
Ethylbenzene, 17.5	5	0	0	0	0
	10	+0.1	+0.2	+0.2	0
	20	0	0	0	0
Benzoic Acid, 10	5	-0.1	0	+0.3	+0.7
	10	0	+0.2	+0.2	0
	20	-0.2	+0.2	-0.2	+0.2
Phenol, 13.5	5	>4.0	-	-	-
	10	>8.1	-	-	-
	20	>16.2	-	-	-
m-Cresol, 20	5	>4.0	-	-	-
	10	>8.1	-	-	-
	20	>16.2	-	-	-
Hydroquinone, 10	5	>4.0	>4.0	-	-
	10	7.9	>8.1	-	-
	20	10.8	11.6	-	-
Aniline, 10	5	>4.0	-	-	-
	10	>8.1	-	-	-
	20	>16.2	-	-	-
Dimethylamine, 20	5	>4.0	-	-	-
	10	>8.1	-	-	-
	20	10.7	-	-	-
Nitrobenzene, 10	5	+0.2	0	+0.2	+0.3
	10	+0.2	-0.4	+0.2	+0.2
	20	+0.3	+0.2	+0.1	+0.8

*NOTE: Negative sign indicates greater residual found in test solution than in control.

methanol*	benzene*
isopropanol*	toluene*
t-butanol*	ethylbenzene*
	benzoic acid
acetone*	nitrobenzene

Those compounds marked with an asterisk (*) were monitored by gas chromatography and exhibited no significant net compound loss (compound control minus test) during the experiments. Moreover, no secondary peaks were observed in any of the chromatograms to indicate the existence of any reaction products. It should be noted that benzene, toluene, and ethylbenzene were rapidly lost from both test and control solutions during mixing. Volatilization was particularly evident in the case of ethylbenzene, as shown in Figure 7.

As evident from Table 7, chlorine was observed to react with phenol, m-cresol, hydroquinone, aniline, and dimethylamine. Phenol (13.5 mg/l), m-Cresol (20 mg/l) and aniline (10 mg/l) completely consumed all of the applied chlorine up to the highest dosage (16.2 mg/l) within a contact time of 0.5 hours. Hydroquinone (10 mg/l) and dimethylamine (20 mg/l) completely removed all of the applied chlorine at the two lower dosages (4.0, 8.1 mg/l) after a 0.5 hour contact time.

Having identified those compounds which were capable of reacting with chlorine, a more detailed experimental program was initiated to provide supplemental information regarding their chlorinating properties. These studies were designed to: (1) further define the chlorine demands of each of the chemicals, (2) establish the contact time required for reaction, and (3) identify, where possible, the formation of specific products. The experimental conditions of this series of experiments were generally similar to those previously described (pH 7.4, 25°C, etc.), however, a wider range of compound and applied chlorine concentrations were utilized.

Phenol was examined at concentrations of 10 and 20 mg/l, with applied chlorine dosages of 20, 50 and 100 mg/l. These data are presented in Table 8 and Figure 8. It is apparent that phenol undergoes a relatively rapid reaction with chlorine during the first 15 minutes of contact, followed by a decreasing rate up to 2 hours contact time. A comparison of control and test chlorine residuals suggests that the reaction is virtually complete after two hours. It is noted that 20 mg/l of phenol completely consumed all of the applied chlorine (50 and 100 mg/l) after 1 hour, and that 10 mg/l of phenol completely exhausted 20 and 50 mg/l of chlorine. For 10 mg/l of phenol, only the application of 100 mg/l of chlorine resulted in a measurable chlorine residual after two hours. Based on this latter test, it may be calculated that phenol exercises a chlorine demand of 6.35 mg Cl_2/mg phenol after two hours contact time. This is equivalent to 8.4 moles of chlorine per

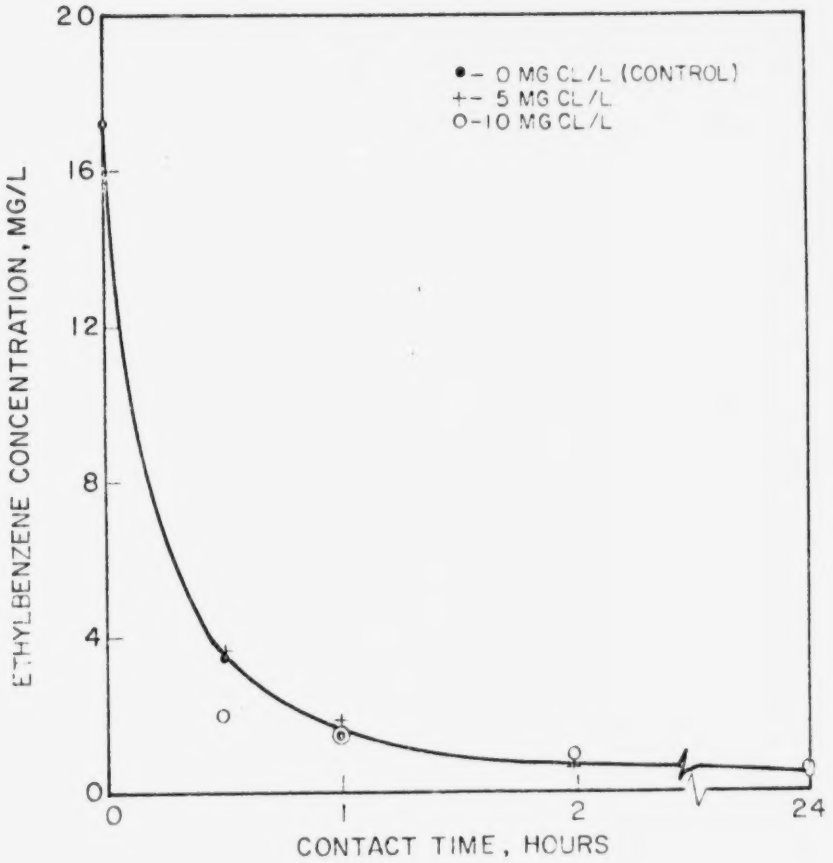


FIGURE 7

VOLATILIZATION OF ETHYLBENZENE DURING CHLORINATION EXPERIMENT

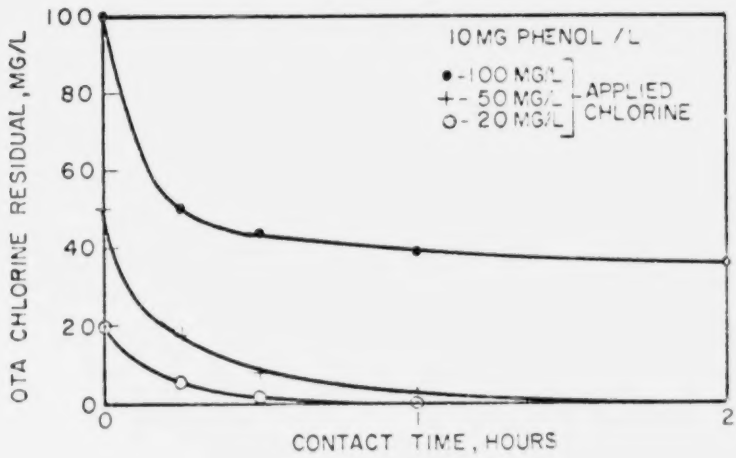


FIGURE 8
REACTION OF CHLORINE WITH PHENOL

mole of phenol. The consumption of chlorine by phenol is also shown in Figure 9, in which the data have been expressed in terms of molar ratios (mmol chlorine per mmol phenol).

TABLE 8

CHLORINATION OF PHENOL

Phenol Concentration (mg/l)	Applied Chlorine (mg/l)	Contact Time (hr)	(OTA) Chlorine Residual (mg/l)	Net Chlorine Demand mg/l	Net Chlorine Demand mmol Cl_2 / mmol phenol
10	20	0.25	6.0	14	1.9
		0.5	1.7	18.3	2.4
		1.0	0	>20	>2.7
		2.0	0	≥20	≥2.7
10	50	0.25	17.8	32.2	4.3
		0.5	8.0	42	5.6
		1.0	2.8	47.2	6.3
		2.0	0	>50	6.6
10	100	0.25	50	50	6.6
		0.5	44	56	7.4
		1.0	39	61	8.1
		2.0	36.5	63.5	8.4
20	50	0.25	4.5	45.5	3.0
		0.5	0	>50	>3.3
		1.0	0	≥50	≥3.3
		2.0	0	≥50	≥3.3
20	100	0.25	12	88	5.8
		0.5	2.5	97.5	6.5
		1.0	0	>100	>6.6
		2.0	0	≥100	≥6.6

An attempt was made to qualitatively identify the products resulting from the reaction of chlorine with phenol, using the gas chromatograph, equipped with dual chromosorb W (HDMS) columns coated with K20M Carbowax TPA. In this experiment, a solution containing 25 mg/l of phenol and 25 mg/l of chlorine was prepared. This selection of concentrations was made to provide an excess of phenol, relative to chlorine, in order to minimize the possibility of direct chlorine oxidation of the phenolic structure. After a contact time of 0.5 hours, a sample was withdrawn for residual chlorine and chromatographic analysis. As anticipated, no chlorine residual was detected. The chromatogram obtained on the sample is shown in Figure 10, which also includes the chromatographic output for a pure phenol solution for comparison. Several additional peaks are evident in the chromatogram of the chlorinated phenol solution.

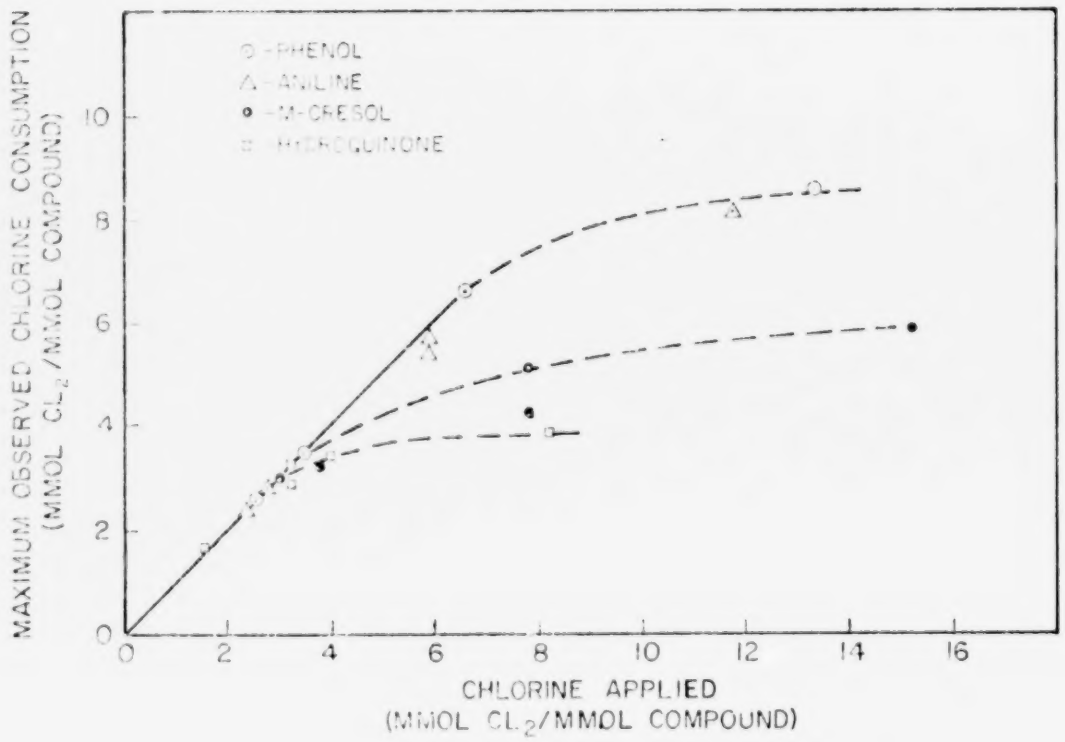


FIGURE 9

MOLAR CHLORINE UPTAKE BY TEST COMPOUNDS

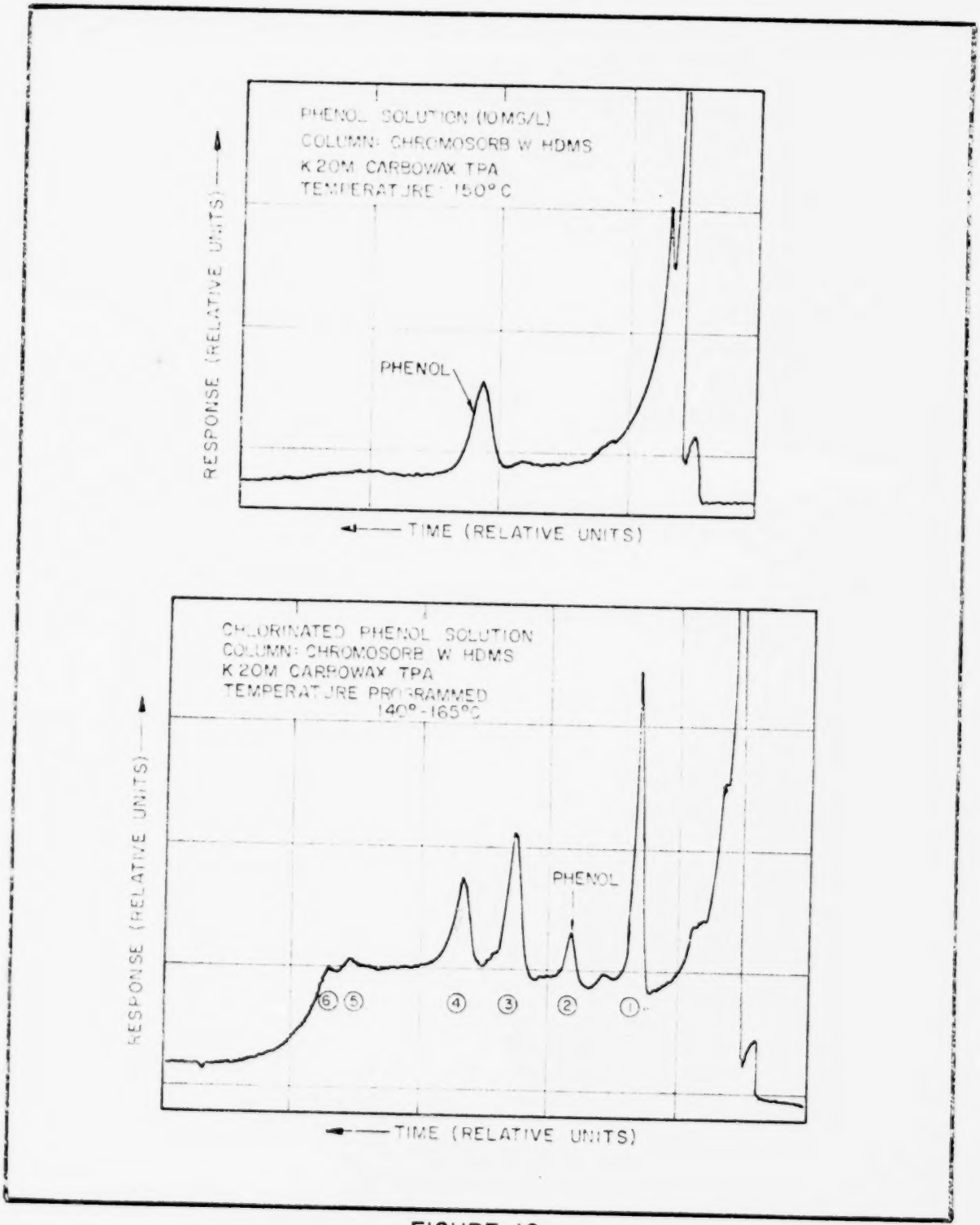


FIGURE 10
CHROMATOGRAMS OF PHENOL
AND
CHLORINATED PHENOL SOLUTIONS

These were subsequently identified, on the basis of relative retention data, as o-chlorophenol (1), 2,6 dichlorophenol (3), 2,4 dichlorophenol (4), 2,4,6 trichlorophenol (5), and p-chlorophenol (6).

The results of similar experiments, using m-cresol as the test compound are shown in Table 9 and Figures 11 and 12. The reaction of chlorine with m-cresol also proceeded quite rapidly in the first 15 minutes, and was essentially complete after two hours contact time. In the case of m-cresol (10 and 20 mg/l), the application of 50 and 100 mg/l of chlorine produced a free chlorine residual after two hours. The maximum observed chlorine uptake (after 2 hours) was computed to be 3.84 mg Cl_2/mg m-cresol (5.9 moles Cl_2/mole m-cresol), corresponding to the solution which initially contained 10 mg/l of m-cresol and 100 mg/l of chlorine. The consumption of chlorine by m-cresol is also shown in Figure 9, and indicates that m-cresol exercises a lower chlorine demand than phenol, on a molar basis.

TABLE 9

CHLORINATION OF m-CRESOL

m-Cresol Concentration (mg/l)	Applied Chlorine (mg/l)	Contact Time (hr)	(OTA) Chlorine Residual (mg/l)	Net Chlorine Demand	
				mg/l	mmol Cl_2 / mmol m-Cresol
10	20	0.25	3.3	16.7	2.5
		0.5	1.5	18.5	2.8
		1.0	0.5	19.5	3.0
		2.0	0.2	19.8	3.0
10	50	0.25	30.8	19.2	2.9
		0.5	30.8	19.2	2.9
		1.0	28.3	21.7	3.3
		2.0	17.0	33.0	5.0
10	100	0.25	81.4	18.6	2.8
		0.5	77.0	23	3.5
		1.0	61.6	38.4	5.9
		2.0	61.6	38.4	5.9
20	50	0.25	16.3	33.7	2.6
		0.5	11.1	38.9	3.0
		1.0	8.0	42	3.2
		2.0	8.0	42	3.2
20	100	0.25	61.6	38.4	2.9
		0.5	58.2	41.8	3.2
		1.0	56.6	43.4	3.3
		2.0	46.0	54.0	4.1

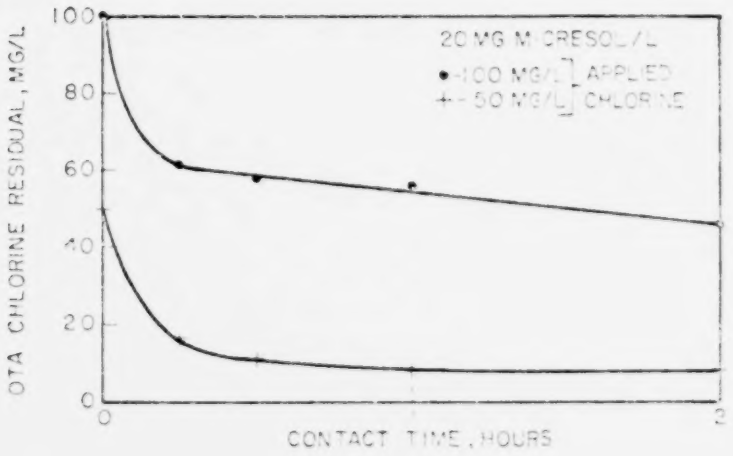


FIGURE 11
REACTION OF CHLORINE WITH M-CRESOL

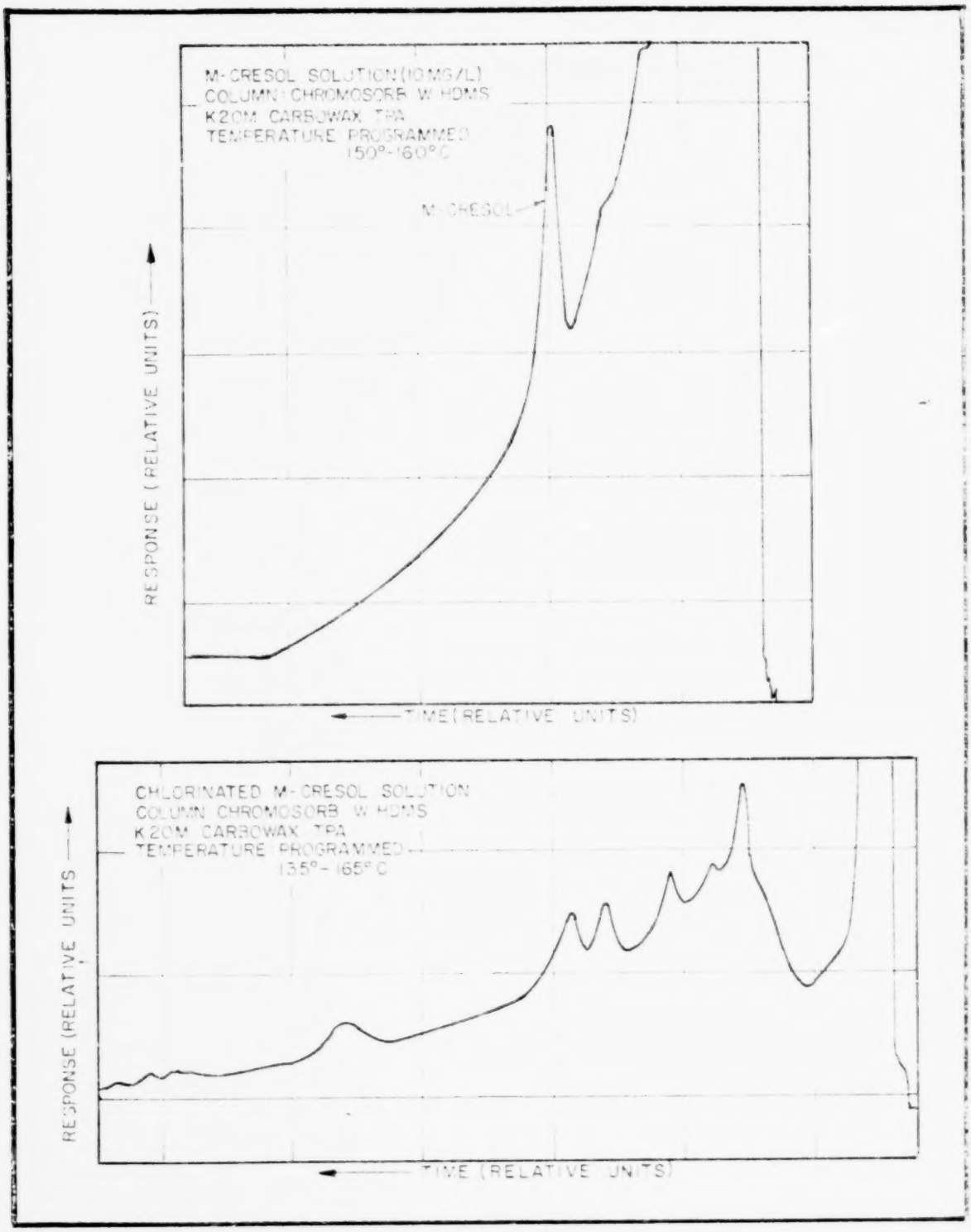


FIGURE 12
CHROMATOGRAMS OF M-CRESOL
AND
CHLORINATED M-CRESOL SOLUTIONS

Chromatograms obtained for m-cresol and a chlorinated solution of m-cresol are shown in Figure 12. Several additional peaks are evident for the latter sample, reflecting the formation of a complex mixture of products. None of these species could be positively identified, due to the lack of commercially available chlorine-substituted cresols. However, it is probable that the products constitute a mixture of chloro-m-cresols and oxidized forms.

The reaction of chlorine with hydroquinone was studied next, using compound concentrations of 10 and 25 mg/l, with nominal applied chlorine dosages of 10, 20, 25, and 50 mg/l. The data for this experiment are presented in Table 10, and Figure 13. As in the case of phenol and m-cresol, a rapid initial reaction was observed, followed by a declining rate of chlorine uptake. The reaction appeared to be complete after two hours. A maximum chlorine demand of 2.37 mg Cl_2/mg hydroquinone (3.9 moles/mole) was observed under the test conditions. (The molar consumption of chlorine is plotted in Figure 9, and shows a lower chlorine demand by hydroquinone, relative to phenol.)

TABLE 10

CHLORINATION OF HYDROQUINONE

Hydroquinone Concentration (mg/l)	Applied Chlorine (mg/l)	Contact Time (hr)	(OTA) Chlorine Residual (mg/l)	Net Chlorine Demand	
				mg/l	mmol Cl_2 / mmol Hydroq.
10	10.4	0.25	0	≥10.4	≥1.6
		0.5	0	≥10.4	≥1.6
		1.0	0	≥10.4	≥1.6
		2.0	0	≥10.4	≥1.6
10	20.8	0.25	6.1	14.7	2.3
		0.5	4.75	16.05	2.5
		1.0	2.75	18.05	2.8
		2.0	1.8	19.0	2.9
10	26	0.25	9.8	16.2	2.5
		0.5	10.0	16.0	2.5
		1.0	5.5	20.5	3.2
		2.0	4.5	21.5	3.3
10	52	0.25	30.8	21.2	3.3
		0.5	32.7	19.3	3.0
		1.0	30.0	22.0	3.4
		2.0	28.3	23.7	3.7
25	52	0.25	9.5	42.5	2.6
		0.5	7.5	44.5	2.8
		1.0	3.5	48.5	3.0
		2.0	1.0	51.0	3.2

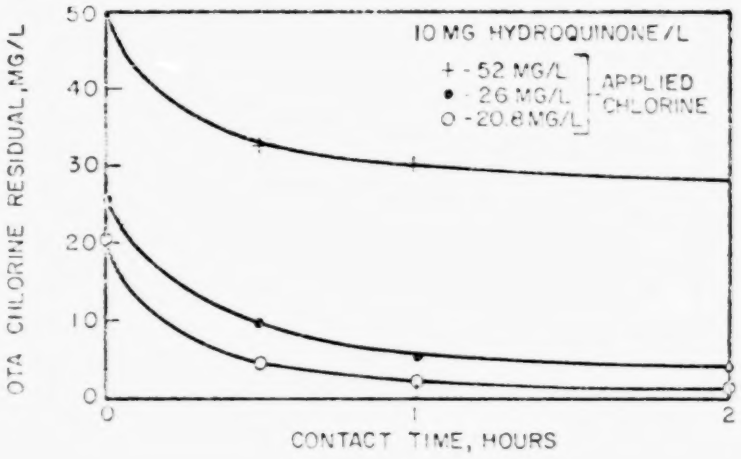
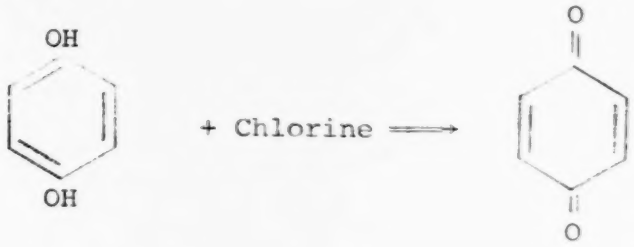


FIGURE 13

REACTION OF CHLORINE WITH HYDROQUINONE

In the absence of an applicable chromatographic procedure, the use of ultraviolet absorption spectrophotometry for reaction product identification was explored. During the previous studies, it was noted that the addition of chlorine to hydroquinone occasionally resulted in the production of orange colored solutions. This behavior was particularly evident at the higher reactant concentrations. Such phenomena are not uncommon in aqueous solutions of aromatic organic chemicals, and generally are the result of partial oxidation of the ring structure. It was thus postulated that hydroquinone reacts with chlorine, at least partially, to form p-benzoquinone as the primary product:



To test this hypothesis, a separate chlorination experiment was conducted. Ultraviolet spectra were first obtained on buffered (pH 7.4) solutions of both hydroquinone (10 and 20 mg/l), and p-benzoquinone (1, 2, and 5 mg/l), as shown in Figure 14. Note that hydroquinone exhibits absorption maxima at ∿220 nm and ∿288 nm, whereas p-benzoquinone absorbs maximally at ∿245 nm. The UV absorption characteristics of each of the pure compounds are thus sufficiently different to allow their identification in a mixed sample.

A solution containing 10 mg/l of hydroquinone and 20 mg/l chlorine was next prepared. This corresponds to an applied chlorine to hydroquinone molar ratio of approximately 3:1, in correspondence to the previously observed chlorine demand. As in previous studies, the pH was maintained at 7.4, using the phosphate buffer. After a contact time of 10 minutes, a sample was withdrawn for UV analysis. The resulting spectrum is also shown in Figure 14, and demonstrates the rapid formation of p-benzoquinone, as indicated by the appearance of an absorption maximum at ∿245 nm. Correspondingly, the peak at ∿288 nm is observed to diminish, reflecting a substantial decrease in the initial hydroquinone concentration. The peak at ∿245 nm (p-benzoquinone) was found to gradually diminish during subsequent measurements over twenty-four hours. Correspondingly, a broad "shoulder" in the 265 - 295 nm region developed over this same period. The species associated with this absorption was not identified.

Aniline was also re-examined in the series of detailed chlorination experiments. Applied chlorine dosages of 18, 45, and

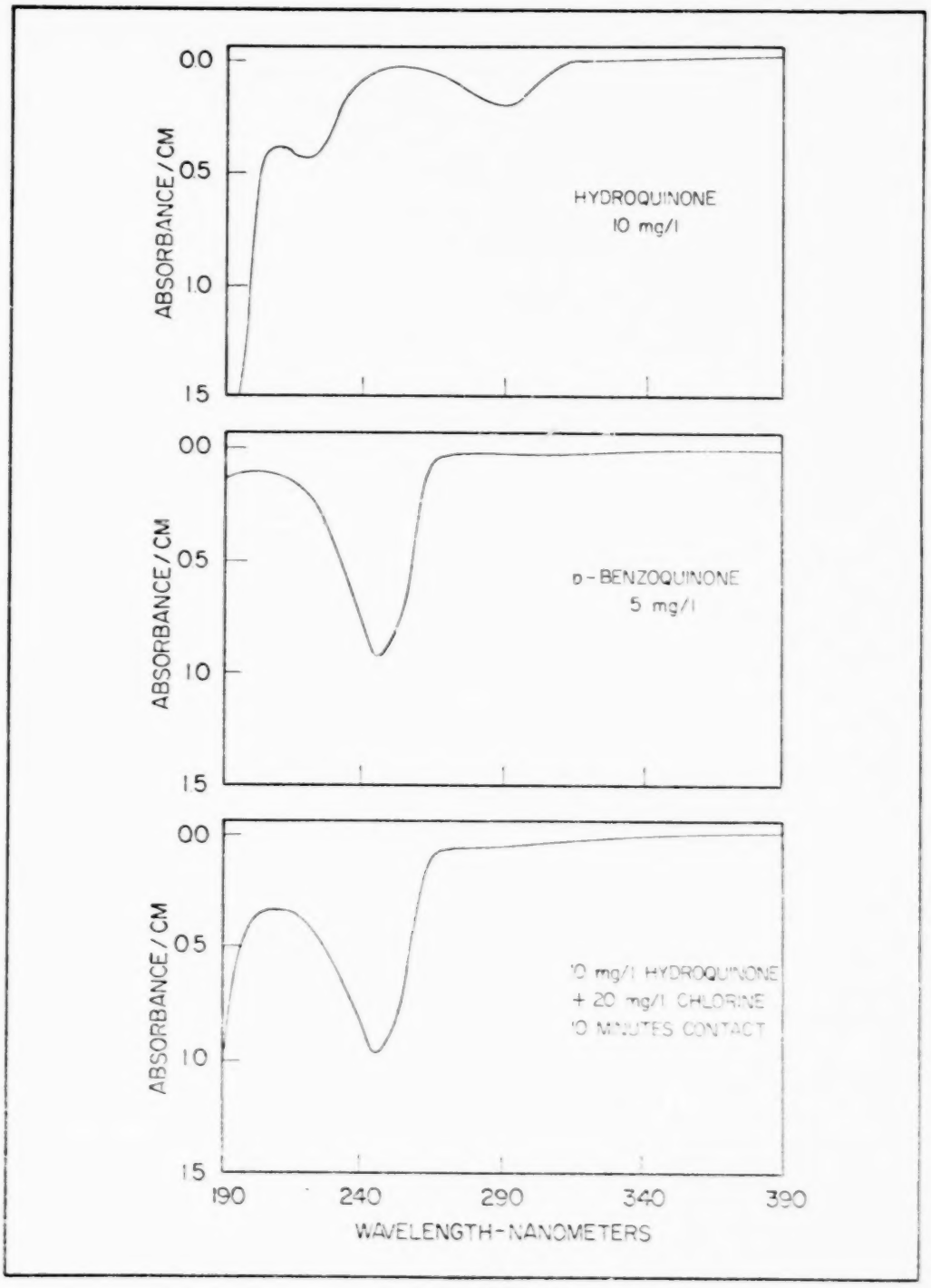


FIGURE 14

ULTRAVIOLET ABSORPTION SPECTRA-REACTION OF CHLORINE WITH HYDROQUINONE

90 mg/l were added to solutions containing 10 and 20 mg/l of aniline. The data derived from these tests are given in Table 11 and Figure 15.

TABLE 11

CHLORINATION OF ANILINE

Aniline Concentration (mg/l)	Applied Chlorine (mg/l)	Contact Time (hr)	(OTA) Chlorine Residual (mg/l)	Net Chlorine Demand	
				mg/l	mmol Cl_2 / mmol Aniline
10	18	0.25	1.75	16.25	2.1
		0.5	0.4	17.6	2.3
		1.0	0.2	>17.8	>2.3
		2.0	0.2	≥17.8	≥2.3
		3.0	0.2	≥17.8	≥2.3
10	45	0.25	27.5	17.5	2.3
		0.5	20	25	3.3
		1.0	14	31	4.1
		2.0	6	39	5.1
		3.0	4	41	5.4
10	90	0.25	58	32	4.2
		0.5	52	38	5.0
		1.0	43	47	6.2
		2.0	34	56	7.3
		3.0	28	62	8.1
20	45	0.25	4.0	41	2.7
		0.5	0.8	44.2	2.9
		1.0	0.6	>44.4	>2.9
		2.0	0.2	≥44.8	≥2.9
		3.0	0.2	≥44.8	≥2.9
20	90	0.25	40	50	3.3
		0.5	30	60	3.9
		1.0	10	80	5.3
		2.0	3.6	86.4	5.7
		3.0	2.6	87.4	5.7

As previously observed for hydroquinone, the appearance of orange colored solutions was evident at the higher applied chlorine levels (50 and 100 mg/l). It may be noted from the figure that aniline also initially undergoes a rapid reaction with chlorine, followed by a declining rate of chlorine uptake. Chlorine consumption was observed to continue over contact times in excess of two hours. The maximum chlorine uptake at three hours was calculated to be 6.2 mg Cl_2/mg aniline (8.1 moles/mole).

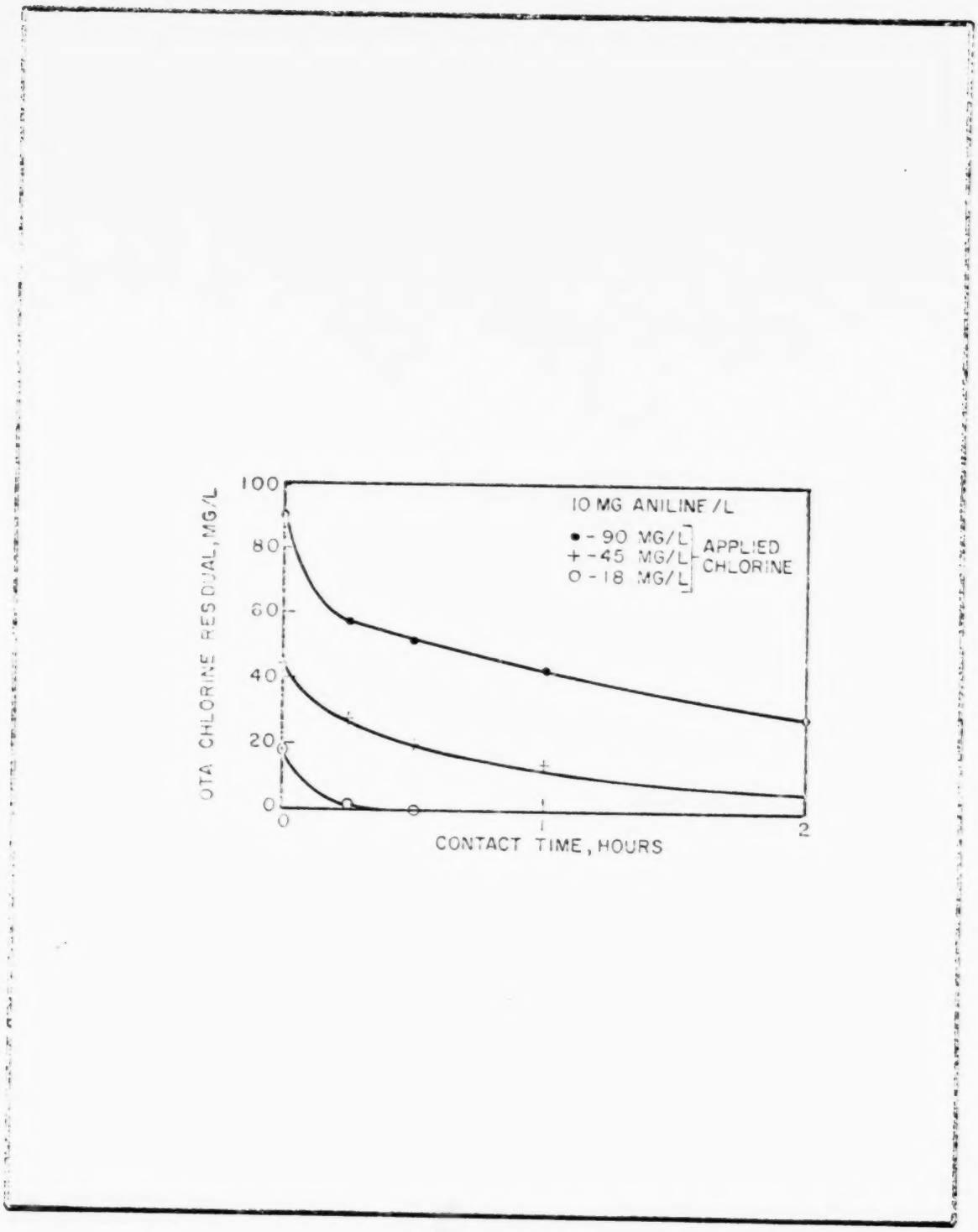


FIGURE 15
REACTION OF CHLORINE WITH ANILINE

In a separate experiment, an attempt was made to gather more information pertaining to the nature of the reaction products of chlorine and aniline. In the absence of reliable chromatographic techniques, ultraviolet spectrophotometric methods were employed for these tests. Solutions were prepared, in accordance with previously described procedures, containing 10 mg/l of aniline, to which chlorine dosages of 5, 10, and 20 mg/l were added. Samples were withdrawn at varying intervals of time for UV spectral scans. Recorded spectra from this sequence of measurements are shown in Figures 16 and 17.

Figure 16 illustrates the effect of chlorine dosage on the ultraviolet spectra of chlorine-contacted aniline solutions. The scans shown were taken on samples after a contact time of one hour. The spectrum indicated by a dashed line in Figure 16 is that of a 10 mg/l aniline solution to which no chlorine had been added, and is included for comparison. Aniline alone exhibits fairly well resolved absorption maxima centered at 230 nm and 281 nm, with no significant absorption above 310 nm. The addition of 5 mg/l of chlorine has the effect of shifting the peak absorbance to about 233 nm in a broad band which extends to 330 nm, with evidence of unresolved inflections, and a shoulder in the region near 285 nm. A dosage of 10 mg/l of chlorine produces a further shift in the absorption peak to about 252 nm. A broad band is again evident which extends to 360 nm and retains the shoulder around 285 nm. The highest chlorine addition (20 mg/l) was observed to cause a further shift of the most prominent peak to about 287 nm. The broadness of the absorption band is also increased, extending to about 370 nm, and showing several unresolved inflections between 220 and 270 nm.

Ultraviolet absorption spectra were also recorded at varying time increments for the 10 mg/l aniline + 10 mg/l chlorine solution. These results are illustrated in Figure 17.

The first scan was obtained after 10 minutes contact time and shows a shift in the absorption peak, producing two poorly-resolved maxima at about 253 nm and 260 nm, with a shoulder centered around 286 nm. After a contact time of one hour, there is no evidence of the dual peaks at 253 nm and 260 nm, but a single broad absorption peak now appears at ~252 nm. Note also that the absorbance of the shoulder has diminished. A final scan, taken at two hours, shows a further decrease in the broad band maximum to about 246 nm. These results are consistent with the data developed in the chlorine uptake studies, and confirm the progressive nature of the aniline-chlorine reaction over contact times in excess of two hours.

Spectra were obtained on dilute aqueous solutions of several chlorine-substituted anilines. The absorption maxima of each compound are as follows:

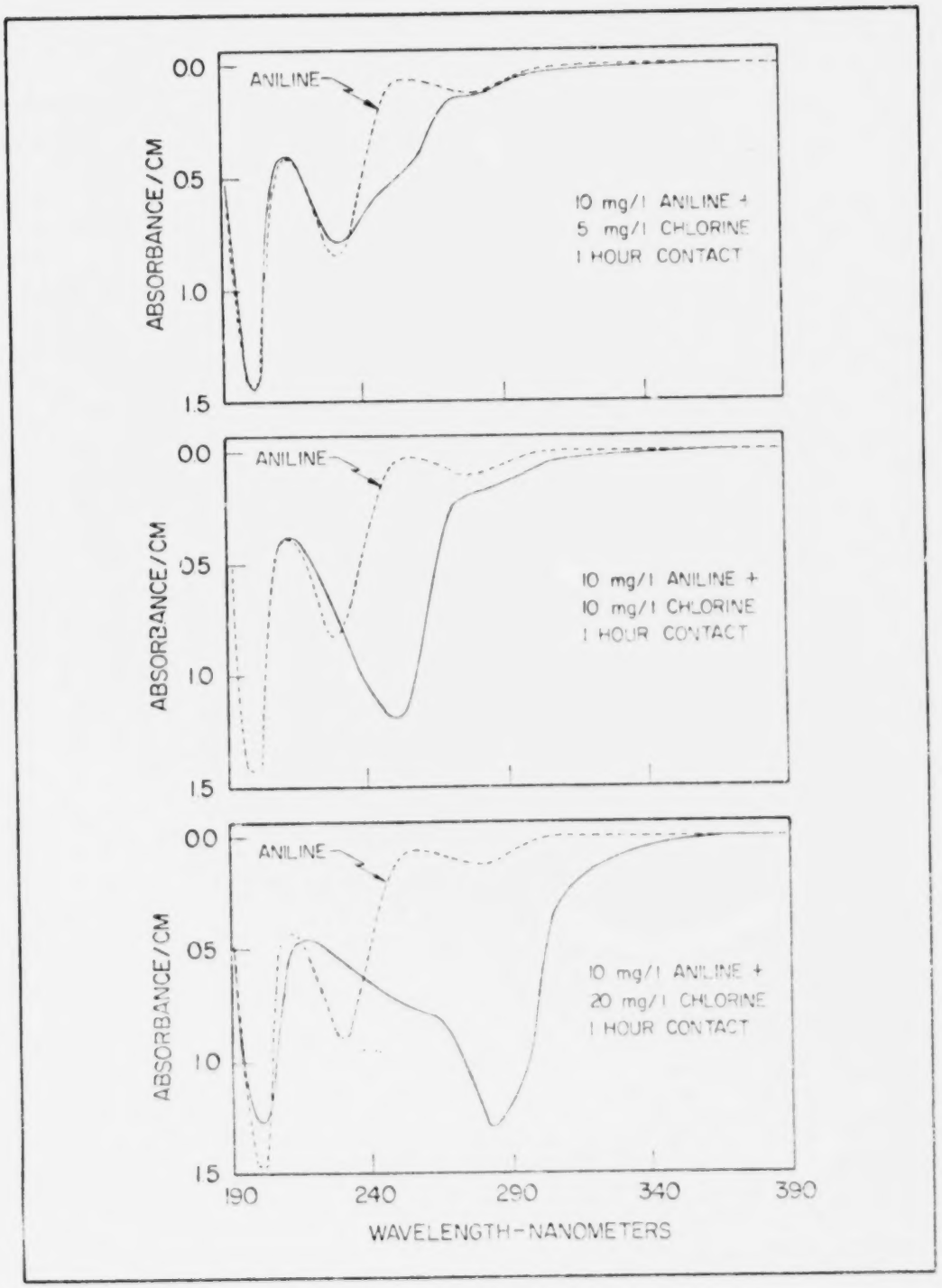


FIGURE 16

ULTRAVIOLET ABSORPTION SPECTRA OF CHLORINE CONTACTED ANILINE SOLUTIONS: EFFECT OF CHLORINE DOSAGE

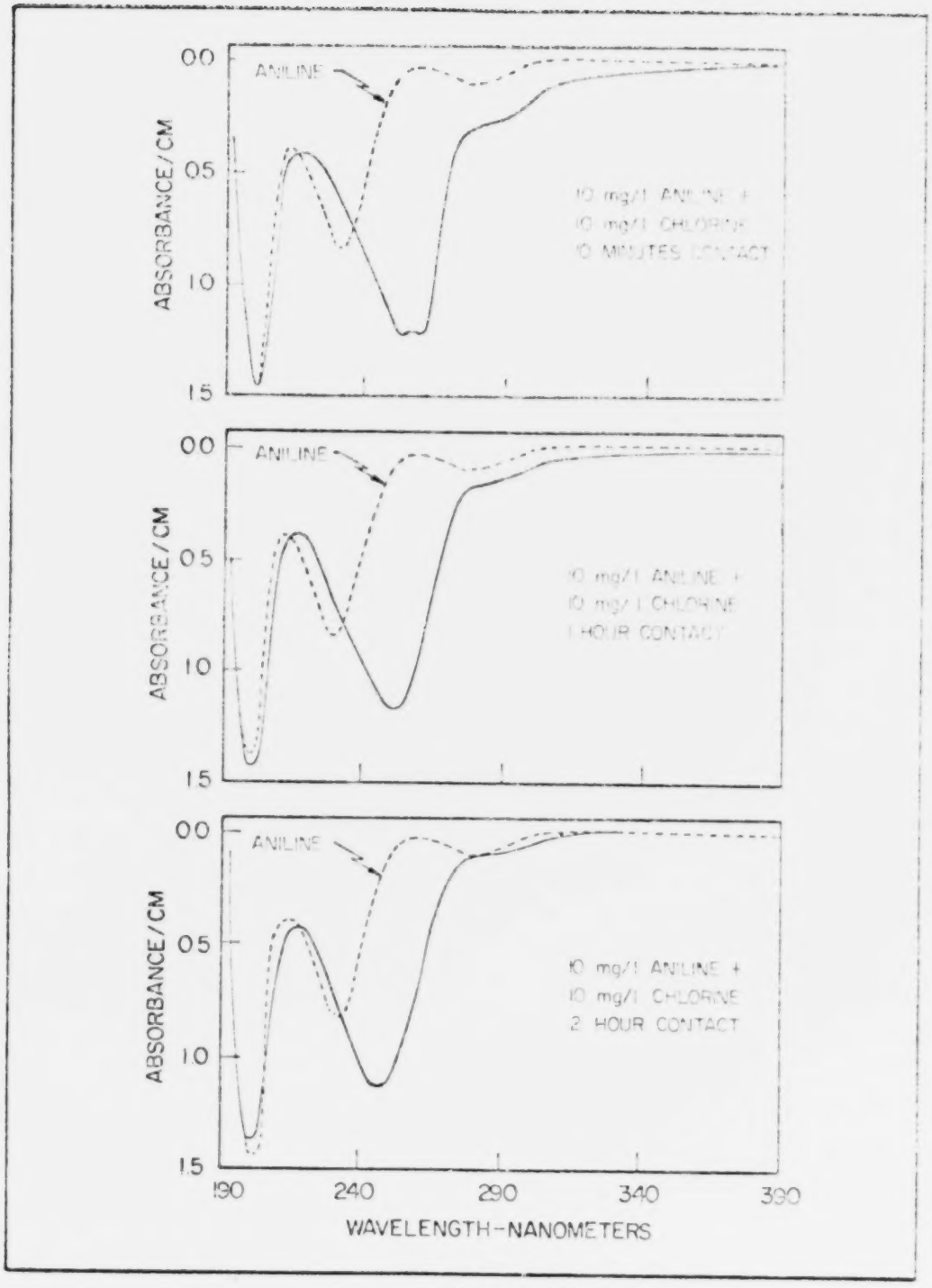


FIGURE 17

ULTRAVIOLET ABSORPTION SPECTRA OF CHLORINE CONTACTED ANILINE SOLUTIONS: EFFECT OF CONTACT TIME

Compound	λ max E-band (nm)	λ max B-band (nm)
aniline	230	281
o-chloroaniline	232	286.5
p-chloroaniline	238.5	293
2,4-dichloroaniline	240	298
2,6-dichloroaniline	228-236 (broad)	291
2,4,6-trichloroaniline	242	304

It is evident that chlorine substitution on the aniline structure promotes a bathochromic shift in the wavelengths of maximum absorption. This effect is enhanced with increasing substitution and is more pronounced with para-substitution, than with ortho-substitution. A comparison of Figure 16 with the data given above reveals that none of the spectra obtained on the pure chloroanilines can be precisely matched with those recorded for the chlorinated aniline solutions. Although there may be some indication of o-chloroaniline formation at the 5 mg/l level of applied chlorine (as evidenced by absorption at ∿233 nm and ∿285 nm), it is apparent that a highly variable and complex mixture of products is formed. The nature and distribution of these reaction products are influenced both by chlorine dosage, and by contact time. The progressive shift of the absorption maximum to higher wavelengths and the appearance of a broad band with increasing chlorine dosage suggests the formation of a mixture of chlorine-substituted anilines. In addition to the formation of chloroanilines, it is also possible that some degree of ring oxidation proceeds simultaneously with ring substitution.

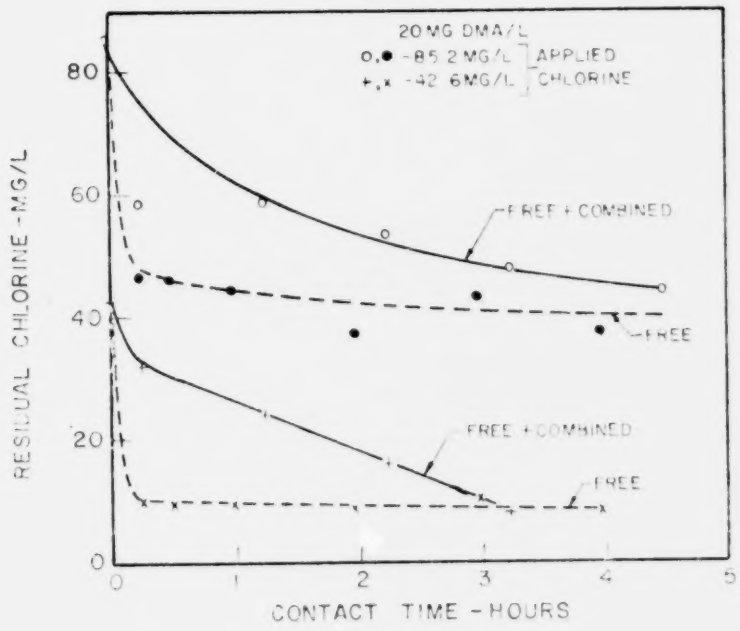
The final series of detailed chlorination experiments was devoted to the re-examination of the chlorine-dimethylamine reaction. Dimethylamine concentrations of 20 mg/l and 100 mg/l were employed in these tests, with nominal applied chlorine levels ranging between 20 and 150 mg/l. Samples were taken at varying time intervals for free residual chlorine analysis (by the OTA procedure) and for total chlorine residual (by iodimetric titration). The results of these investigations are given in Table 12 and Figure 18.

It is apparent from the data that: (1) the addition of chlorine to dimethylamine solutions results in a rapid consumption of free chlorine; and (2) the total chlorine residual, including a combined fraction formed in the initial stages of the reaction, gradually diminishes with time. The initial reaction appeared to be complete after a contact time of 15 minutes or less, whereas the loss of combined residual chlorine was observed to proceed over several hours. The data also indicate that the degree of chlorine uptake is variable,

TABLE 12

CHLORINATION OF DIMETHYLAMINE

DMA Concentration (mg/l)	Applied Chlorine mg/l	Applied Chlorine mmol Cl_2 / mmol DMA	Contact Time (hr)	Chlorine Residual (mg/l) Tot.	Chlorine Residual (mg/l) Free	Net Free Chlorine Demand mg/l	Net Free Chlorine Demand mmol Cl_2 / mmol DMA
20	21.3	.68	0.25	13.3	0.75	20.55	
			0.5	-	0.3	21	
			1.0	8.0	0.3	21	21 .67
			2.0	5.3	0.25	21.05	
			3.0	0	0.25	21.05	
			4.0	0	0.2	21.1	
20	42.6	1.35	0.25	31.9	10.0	32.6	
			0.5	-	10.0	32.6	
			1.0	23.9	9.5	33.1	33.1 1.05
			2.0	15.9	9.0	33.6	
			3.0	8.0	10.0	32.6	
			4.0	2.6	8.5	34.1	
20	85.2	2.71	0.25	58.5	46.5	38.7	
			0.5	-	46.5	38.7	
			1.0	58.5	44.5	40.7	41.5 1.32
			2.0	53.2	37.5	44.7	
			3.0	47.9	43.5	41.7	
			4.0	43.9	37.5	44.7	
100	75	.48	0.5	62.4	0.1		
			1.0	49.4	0		
			2.0	27.3	0	75	.48
			3.0	11.7	-		
			5.0	9.1	-		
100	112.5	.715	0.5	91.0	0.2		
			1.0	55.9	0		
			2.0	37.7	0	112.5	.715
			3.0	18.2	-		
			5.0	7.8	-		
100	150	.95	0.5	119.6	1.2		
			1.0	65.0	.55		
			2.0	42.9	.2	150	.95
			3.0	19.5	-		
			5.0	7.8	-		



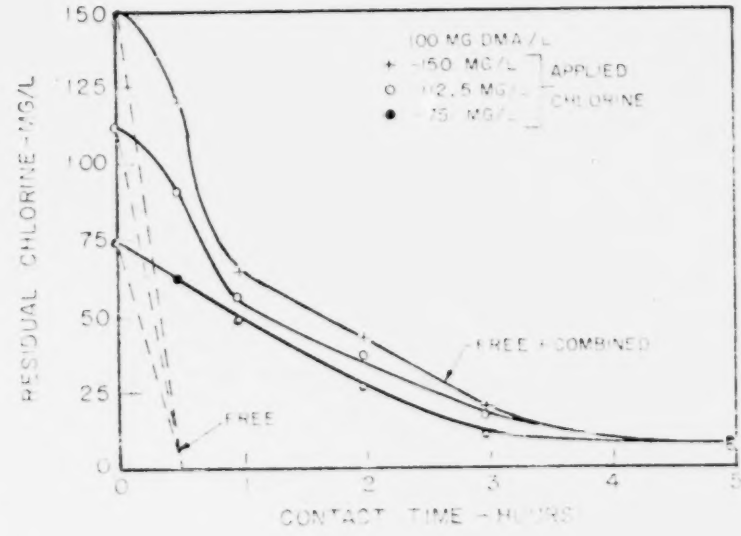


FIGURE 18

REACTION OF CHLORINE WITH DIMETHYLAMINE

and increases with increasing applied chlorine dosage. This is evident from Table 12, which shows both the molar consumption of chlorine per mole of DMA, and the applied chlorine to DMA molar ratio. Note that dimethylamine readily consumes at least 1 mole of chlorine per mole of DMA. At higher chlorine applications, chlorine demands greater than unity were observed, but a free chlorine residual was maintained. A maximum chlorine uptake of approximately 1.3 moles Cl_2/mole DMA was noted, at an applied chlorine to DMA molar ratio of about 2.7.

A rigorous characterization of the nature of the products of the reaction of DMA with chlorine could not be made, due to the lack of suitable analytical tests. In preliminary experiments, it was found that DMA does not respond to the chemical oxygen demand test. Although it was determined that DMA could be detected in aqueous solutions by gas chromatography, the response was found to be non-quantitative. Additionally, dimethylamine was observed to be insensitive to ultraviolet spectrophotometric analysis. These difficulties precluded the positive identification of any products of the DMA-chlorine reaction.

Discussion of Results - Chlorination Studies

One of the major objectives of the experimental program of Phase I was the determination of the ability of each of the selected chemicals to react with chlorine. A corollary, but nonetheless vital requirement, was the establishment of testing procedures by which this characterization could be achieved. It was recognized, a priori, that virtually all of the test compounds could, under the appropriate conditions, react with chlorine. In fact, halogenation reactions are among the most important in the synthesis of complex organic chemicals. However, many of these reactions do not proceed to any appreciable extent, unless rigorous chemical or physical driving forces are applied (elevated temperature, pressure, catalysis, etc.). Obviously, such conditions would not be encountered in conventional effluent chlorination practice. The selection of experimental parameters was thus constrained to a rather narrow range of ambient temperatures, pH values near neutrality, and dilute aqueous solutions of reactants. It was anticipated that some of the test compounds, which are commonly employed in industrial halogenation processes, would be essentially unaffected by the levels of chlorine commonly applied to effluents. This assumption was confirmed by the experimental data, which indicated that only five of the fourteen original test chemicals were observed to react with chlorine to any substantial degree.

None of the alcohols tested (methanol, isopropanol, t-butanol) exhibited any tendency to exercise a chlorine demand. Although many alcohols may undergo halogenation, the reactant

employed is usually a hydrogen halide (HX). Moreover, the reaction is commonly achieved at elevated temperatures, in the presence of a catalyst or strong acid, or in a nonaqueous gas phase. As such, it is unlikely that a dilute aqueous solution of chlorine would affect any of the alcohols examined in this study.

No significant chlorine uptake was observed in the case of acetone. At first inspection, this result seemed anomalous, as it is known that saturated ketones and aldehydes will generally undergo halogenation in aqueous solutions. It has been reported that the rate of halogenation of acetone is dependent on the acetone concentration, and is significantly increased by either acidic or alkaline catalysis, as represented by the following expression[6]:

$$V = [(CH_3)_2CO][6 \times 10^{-9} + 5.6 \times 10^{-4}[H^+] + 7[OH^-]]$$

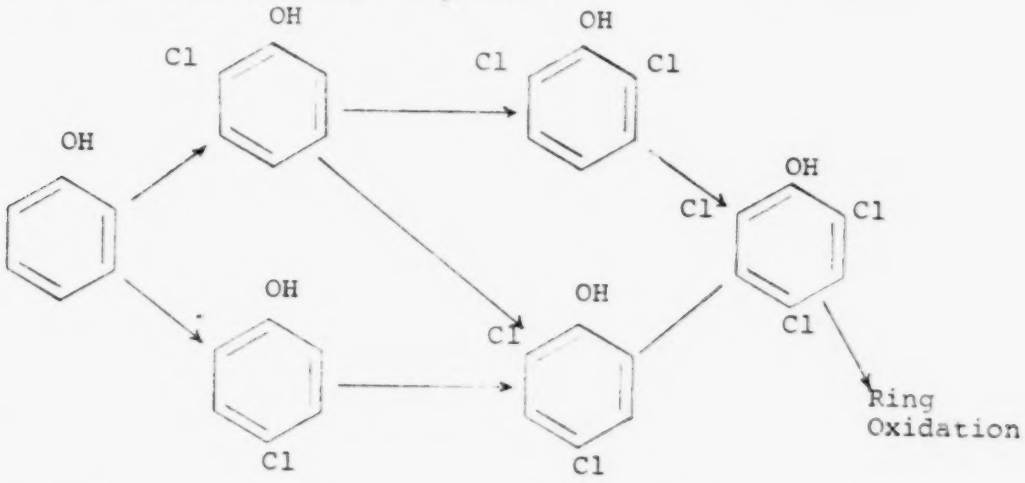
where V is the reaction rate in moles/liter/second, and the bracketed terms correspond to the molar concentrations of the respective species. Using this expression, the calculated rate of chlorination of 20 mg/l of acetone at pH 7.4 is approximately 2.2×10^{-3} mmol/liter/hour, or about 0.6% per hour. In the present study, the measurement of this low rate was not compatible with the precision of the chromatographic analysis of acetone nor the OTA method for chlorine. Consequently, a chlorine uptake by acetone could not be observed, under the test conditions.

Benzene, and its derivatives, toluene, ethyl benzene, and benzoic acid, also showed no evidence of reactions with chlorine. Again, halogenations of benzenes are rather commonplace in the chemical process industry, but a catalyst is usually required to achieve this substitution.

It is thus unlikely that benzene or the alkyl benzenes could react with chlorine in the dilute aqueous solutions considered in this study. Benzoic acid and nitrobenzene were considered to be even less reactive than benzene, due to the electron-attracting and ring-deactivating effects of the -COOH and $-NO_2$ groups.

In contrast to benzene and the derivatives of benzene described above, phenol was determined to be quite reactive to chlorine, in dilute aqueous solution. This result is by no means surprising, since the presence of taste and odor-causing chlorophenols in certain chlorinated wastewater effluents has been demonstrated by many investigators[7,8]. The high reactivity of phenol is attributable to the ring-activating electron-releasing properties of the -OH functional group.

The nature of the activating group is such that halogen substitution in aqueous solution is preferentially favored in the ortho- and para- positions, with respect to the -OH group. This fact has also been confirmed by several researchers[9,10]. Burttschell et.al.[9] proposed the following reaction scheme to describe the chlorination of phenol:



Note that the reaction proceeds by the stepwise substitution of the 2, 4, and 6 (ortho- and para-) positions of the aromatic ring. Ring oxidation follows the formation of 2,4,6-trichlorophenol. It is probable that the reactions proceed simultaneously, as well as sequentially, resulting in the formation of a complex mixture of chlorophenols and the oxidation products. The nature and distribution of these products are doubtlessly affected by such parameters as reaction time, proportions of reactants, pH, temperature, and other conditions. Lee[10] has indicated that the maximum rate of chlorination occurs between pH 7 and pH 9.

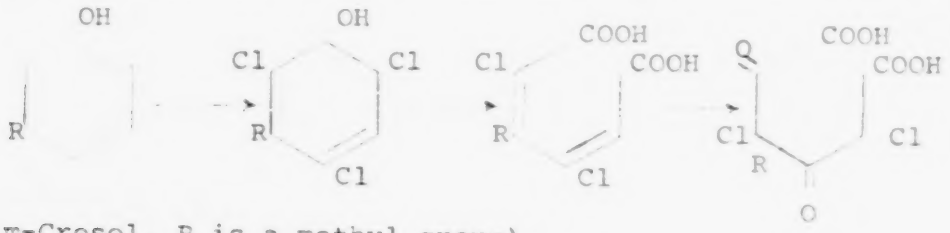
In the present study, the formation of all of the chlorophenols mentioned above was confirmed using gas chromatographic techniques. The appearance of several unidentified peaks in the chromatograms also suggested the existence of oxidized forms. Additional evidence for ring oxidation may be derived from the fact that phenol exhibited a chlorine demand in excess of the amount required to completely form trichlorophenol. A maximum chlorine uptake of 8.4 moles Cl_2/mole phenol was observed in this study, compared with a stoichiometric requirement of 3 moles/mole for trichlorophenol formation.

Meta-cresol, a methyl-substituted phenol, exhibited chlorinating properties similar to phenol. This is consistent with the structural similarities (and therefore, reactivities) of the two compounds. A moderately activating methyl group in the meta position should serve to reinforce the pattern of chlorine

substitution observed for phenol, i.e., by directing to the ortho- and para- (2,4,6) positions. Upon the addition of chlorine, it would therefore be expected that a complex mixture of chlorine-substituted and oxidation products would form.

Evidence for this behavior is manifested by an observed chlorine demand of 5.9 moles/mole, which is considerably in excess of the theoretical requirement to produce a tri-substituted m-Cresol, and by the appearance of at least eight peaks in the chromatogram of a chlorinated m-Cresol solution. Unfortunately, no qualitative identification of the products of chlorination could be attempted, due to the lack of commercially available chloro-m-Cresols.

In a study devoted to the oxidation of phenols by chlorine, Eisenhauer[11] suggested the following probable sequence of reactions resulting from the chlorination of a substituted phenol.



(for m-Cresol, R is a methyl group).

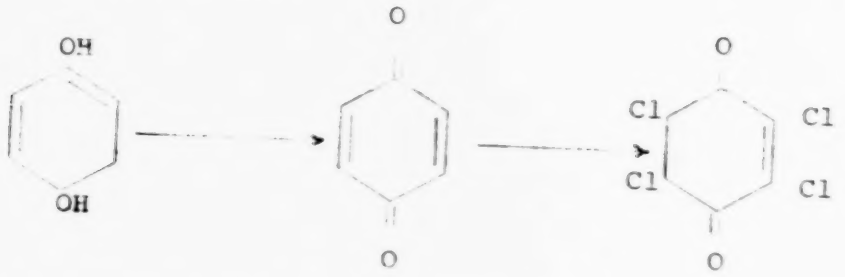
The formation of non-aromatic oxidized products, such as carboxylic acids, at higher levels of applied chlorine, was postulated on the basis of observed shifts in ultraviolet absorption spectra. This hypothesis is consistent with the relatively high chlorine demands noted for both m-Cresol and phenol.

Hydroquinone is a dihydric phenol, containing -OH groups in the 1,4 positions. This configuration of two highly activating substituents should even further magnify the destabilization of the ring structure. Indeed, hydroquinone may be readily converted to p-benzoquinone by a variety of oxidizing agents.

As previously described, the oxidation of hydroquinone to p-benzoquinone by chlorine was confirmed in this study, using ultraviolet spectrophotometric procedures. Apparently, this is not the only reaction, since a maximum chlorine demand of 3.7 moles Cl_2/mole was observed, which is greater than the theoretical requirement for simple oxidation to p-benzoquinone. Moreover, when a solution containing an applied chlorine to hydroquinone molar ratio of about 1.5 was analyzed after ten minutes contact time, it was found to contain approximately one-half of concentration of p-benzoquinone that would have

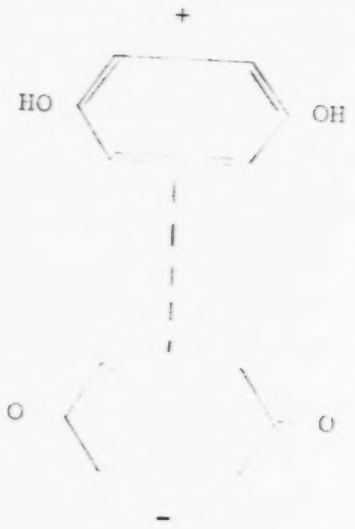
been expected if complete conversion had occurred. These observations suggest the formation of other substituted or oxidized products resulting from the reaction of chlorine with hydroquinone.

During the preliminary experiments with hydroquinone, the formation of a tetrasubstituted quinone, chloranil, was considered as a possibility:



However, attempts to isolate chloranil were unsuccessful, due to its extremely low solubility in water. It is now believed that chloranil is probably not a product of the hydroquinone-aqueous chlorine reaction, since the usual method of preparation of chloranil involves the use of hydrogen chloride.

The possibility also exists for the formation of a charge-transfer (π) complex of p-benzoquinone and unreacted hydroquinone of the form:

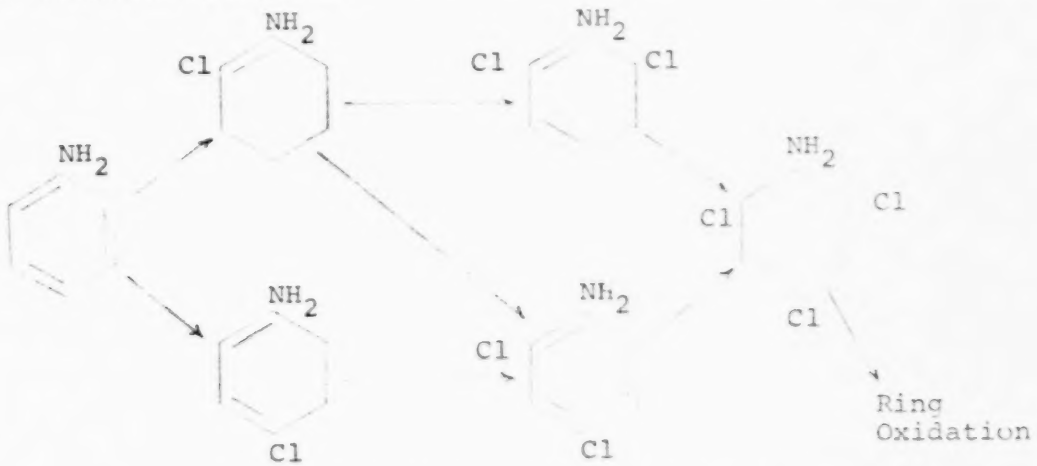


The formation of this species is generally accompanied by the appearance of a dark-colored solution and a bathochromic shift

in the ultraviolet absorption spectrum[7]. Phenomena of this type were observed at the higher reactant (hydroquinone and chlorine) concentrations, which could possibly explain the non-stoichiometric production of p-benzoquinone, as described above.

In summary, it appears that additions of chlorine to dilute aqueous solutions of hydroquinone results in the formation of p-benzoquinone as a primary product. This compound is believed to persist either as a separate species, as part of the quinhydrone complex, or combinations of the two. Further additions of chlorine, resulting in chlorine uptakes of at least 3.7 moles Cl_2/mole are likely to produce further oxidized products, such as carboxylic acids.

Aniline, by virtue of a strongly activating functional group ($-NH_2$), should also be expected to possess chlorinating properties similar to phenol. It is known that bromine reacts rapidly with aniline to form 2,4,6-tribromoaniline in high yield, and it has been reported that, depending on the nature of the oxidizing agent, aniline may be oxidized to either nitrobenzene, p-benzoquinone, or other products of ring cleavage[7]. It may thus be postulated, by analogy to phenol, that aniline may undergo the following reaction steps when contacted with aqueous chlorine solutions:



Indirect evidence for this reaction scheme is shown in Figure 9, in which the observed chlorine consumption by aniline, on a molar basis, is noted to be quite similar to phenol.

It is also evident from the experimental data that the chlorination of aniline results in the generation of highly complex reaction products. Ultraviolet spectra taken on lightly chlorinated (0.5 mg Cl_2/mg aniline) solutions (see Figure 16) suggest the production of a mixture of chloroanilines.

Higher applied chlorine levels (1-2 mg Cl_2/mg) seem to promote the formation of oxidized species. Note the UV absorption spectra obtained on a 10 mg/l aniline solution, to which 10 mg/l of chlorine had been added. The disappearance of peaks in the region of 230 nm and 280 nm indicate a loss of aromatic structure, normally associated with these absorptions. Moreover, the appearance of a single broad band suggests the formation of either a partially oxidized non-aromatic ring or a conjugated straight chain molecule that resulted from ring scission.

There is also evidence for a continuing sequence of reactions that proceeds even after a complete loss of free chlorine residual. For the 1:1 solution described above, no free chlorine residual was detected after a one-half-hour contact time, yet the UV absorption pattern continued to change over a two-hour period. This may be indicative of the production of intermediate species, which may subsequently undergo structural rearrangement. The resulting broad band at 246 nm was found to persist during UV scans taken over eighteen hours. The position and the shape of this absorption is quite similar to that observed for p-benzoquinone, a possible product of aniline oxidation.

Dimethylamine, a secondary aliphatic amine, is also known to participate in halogen reactions, leading to the formation of mono-N-haloamines.

In a previous study devoted to the rate of formation of chloramines, Weil and Morris[12] indicated that only a monochloro derivative results from the reaction of chlorine and dimethylamine, corresponding to the following stoichiometric representation:

$$CH_3\text{-}\overset{H}{N}\text{-}CH_3 + Cl_2 \rightarrow CH_3\text{-}\overset{Cl}{N}\text{-}CH_3 + HCl$$

It is evident from this reaction that the stoichiometric chlorine requirement is one mole per mole of DMA. The results from the present study generally confirm this stoichiometry, however, in cases of higher applied chlorine doses, chlorine demands greater than one mole per mole were observed.

Marks[13] has stated that the monochloro derivative of dimethylamine (N-chloro-DMA) should be amenable to analysis by the acid iodometric titration procedure, which was utilized in the supplementary DMA chlorination experiments. Indeed, an iodometric chlorine residual in excess of the free chlorine residual (OTA) was observed in the early stages of the reaction, suggesting the initial formation of some chloro-DMA compound, probably N-chloro-DMA. The subsequent loss of combined chlorine residual indicates that the compound is either unstable, or is lost from the system by volatilization.

In summary, it has been shown that five of the fourteen original test chemicals were observed to participate in reactions with free chlorine under the moderate conditions associated with conventional effluent chlorination practice. These compounds are: phenol, m-cresol, hydroquinone, aniline, and dimethylamine. In some cases, it has been possible to relate the experimentally determined chlorine-reactivity of these species to their respective molecular structures. For example, of the nine aromatic compounds examined in the study, only those possessing "ring-activating" substituent groups were noted to react with chlorine in dilute aqueous solutions. These considerations should facilitate the preliminary characterization of the potential of other similar species to undergo chlorination reactions.

The information developed in this phase of the study clearly indicates that the application of chlorine to dilute solutions of any of the chlorine-reactive chemicals results in a highly complex mixture of products. It is apparent that the nature and distribution of these products is dependent on a variety of parameters, which include reactant concentrations, pH, and temperature. A list of the probable products of chlorination has been assembled and is presented in Table 13. This tabulation was compiled on the basis of the experimental data generated in this study, supplemented by various literature sources.

One of the objectives of the experimental program of Phase I was the selection of five products of chlorination for subsequent studies. The chemicals initially chosen for further examination were 2,4,6-trichlorophenol, 2,4,6-trichloroaniline, 4-chloro-3-methylphenol, N-chloro-DMA, and chloranil. The original selection was made on the basis of available information relating to probability of formation, chemical stability, and commercial availability, or ease of synthesis.

Specifically, 2,4,6-trichlorophenol was chosen since it had been identified as a product of phenol chlorination, and represented the final reaction step prior to ring oxidation. Its existence had also been demonstrated by previous investigators, who indicated that it was difficult to biologically degrade and was relatively toxic to aquatic organisms[1,2,3].

Trichloroaniline was selected for further study because of the chemical similarity of aniline to phenol, as well as a strong theoretical basis for its existence. Virtually no information is available regarding the behavior of this compound in biological systems.

The only commercially available chlorinated product of m-Cresol was 4-chloro-3-methyl phenol, and as little is known about the products of m-cresol chlorination, this compound was carried into the Phase II studies.

TABLE 13

PROBABLE PRODUCTS OF CHLORINATION

Phenol
o-chlorophenol
p-chlorophenol
2,4-dichlorophenol
2,6-dichlorophenol
2,4,6-trichlorophenol
non-aromatic oxidation products

m-Cresol
2-chloro-3-methylphenol
4-chloro-3-methylphenol
6-chloro-3-methylphenol
2,4-dichloro-3-methylphenol
2,6-dichloro-3-methylphenol
4,6-dichloro-3-methylphenol
2,4,6-trichloro-3-methylphenol
non-aromatic oxidation products

Hydroquinone
p-benzoquinone
non-aromatic oxidation products

Aniline
o-chloroaniline
p-chloroaniline
2,4-dichloroaniline
2,6-dichloroaniline
2,4,6-trichloroaniline
non-aromatic oxidation products

Dimethylamine
N-chloro-DMA
oxidation products

Of the primary test chemicals considered, dimethylamine was judged to be one of the more likely to survive biological treatment intact. Since some evidence existed for the formation of the monochloro derivative, N-chloro-DMA was deemed suitable for further study. Although N-chloro-DMA was not available commercially, it was originally anticipated that its synthesis could be achieved in the laboratory. However, when subsequent studies indicated that N-chloro-DMA was chemically unstable, this species was dropped from further consideration.

Chloranil, although not shown on the list of probable products of chlorination, was initially suspected to be a product of hydroquinone chlorination. As such, it was originally chosen for examination in Phase II. When additional experiments failed to establish the existence of chloranil as a reaction product, p-benzoquinone, a confirmed product of the hydroquinone-chlorine reaction was substituted in the following studies.

SECTION VIII

RESPIROMETER STUDIES - PHASE II

As a result of the Phase I studies, 5 compounds were selected for further study in Phase II. The selected compounds were:

2,4,6-trichlorophenol
4-chloro-3-methyl phenol
chloranil
2,4,6-trichloroaniline
N-chlorodimethylamine

The first portion of Phase II was the conduction of respirometer studies to examine the possible inhibition or toxicity of the selected compounds to an operative biological system.

Experimental Procedure

The respirometer studies were conducted in a modified Warburg apparatus, known as a "Gilson" respirometer. The system differed from a "Warburg" only in the method of pressure difference measurement. The control system was an inocculated synthetic sewage of the composition described in a previous section of the report. The test systems contained the same inocculum and sewage, plus the desired concentration of the particular substrate. In most cases, a substrate concentration range of 1 mg/l to 100 mg/l was studied. For comparative purposes, one experiment was conducted with copper as the test substrate. The inhibitory influence of copper is well documented, and was included to provide a basis for comparison of effects.

Study Results

The study results are presented graphically in Figures 19 through 24. Figure 19 shows that 2,4,6-trichlorophenol does not inhibit reaction at 1 or 10 mg/l. In Figure 20, data for 50 mg/l and 100 mg/l 2,4,6-trichlorophenol, and for 1 mg/l and 10 mg/l copper are presented. All reactions presented in the plot show significant inhibition of the biological reaction. Figures 21 and 22 provide information on 4-chloro-3-methyl phenol. The substrate is mildly inhibitory at lower concentration (10 mg/l), strongly inhibitory at 50 mg/l, and apparently toxic at 100 mg/l. Figure 23 presents data for chloranil, which is inhibitory at 10 mg/l. Higher concentrations were not studied because of solubility limitations. The 2,4,6-trichloroaniline data shown in Figure 24 suggests the material is not inhibitory up to 10 mg/l. Solubility considerations precluded investigation at higher concentrations.

N-chlorodimethylamine was originally considered for study but an investigation showed that the compound was unstable, and

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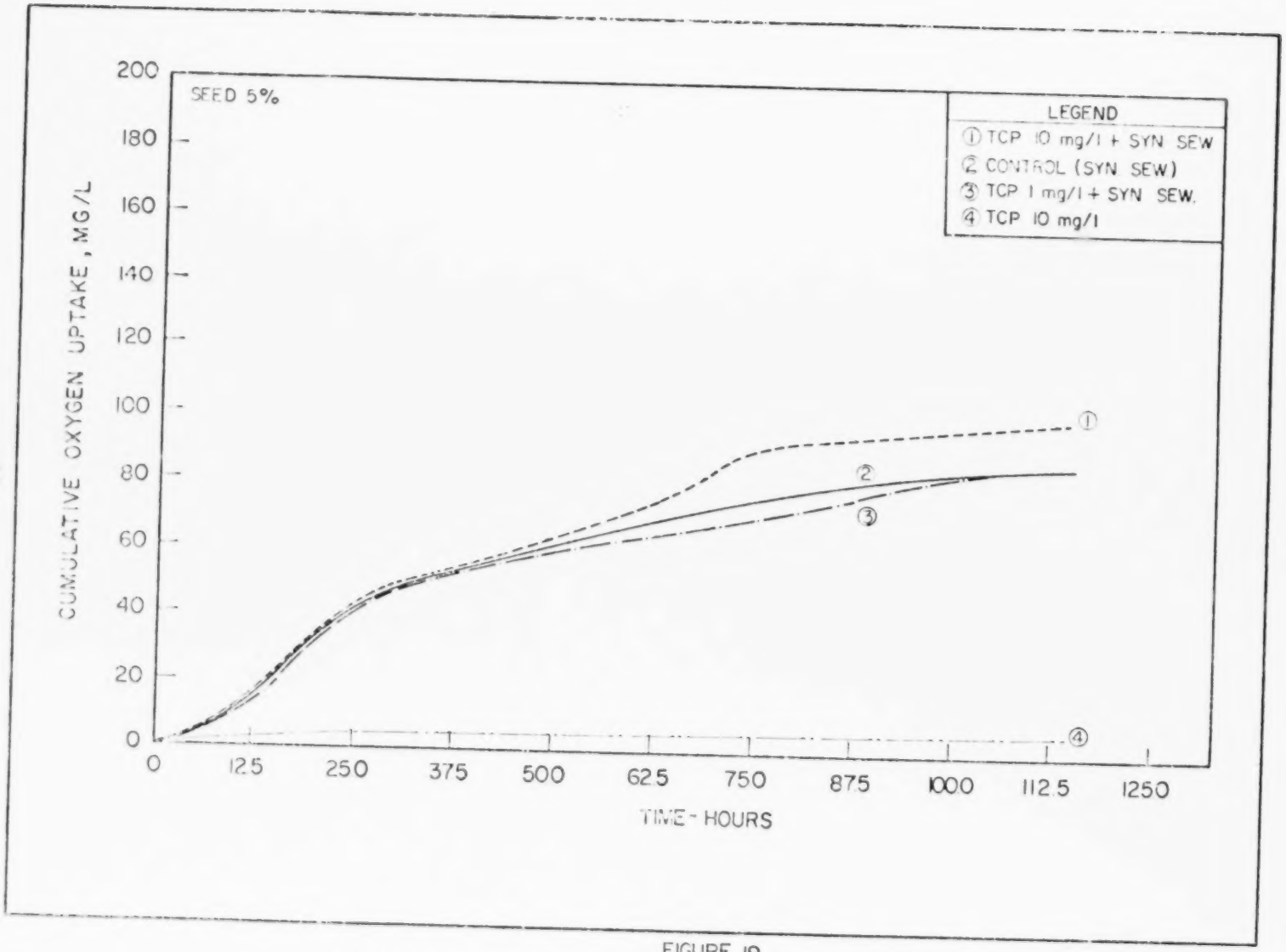


FIGURE 19

EFFECT OF 2,4,6-TRICHLOROPHENOL ON A MIXED MICROBIAL POPULATION

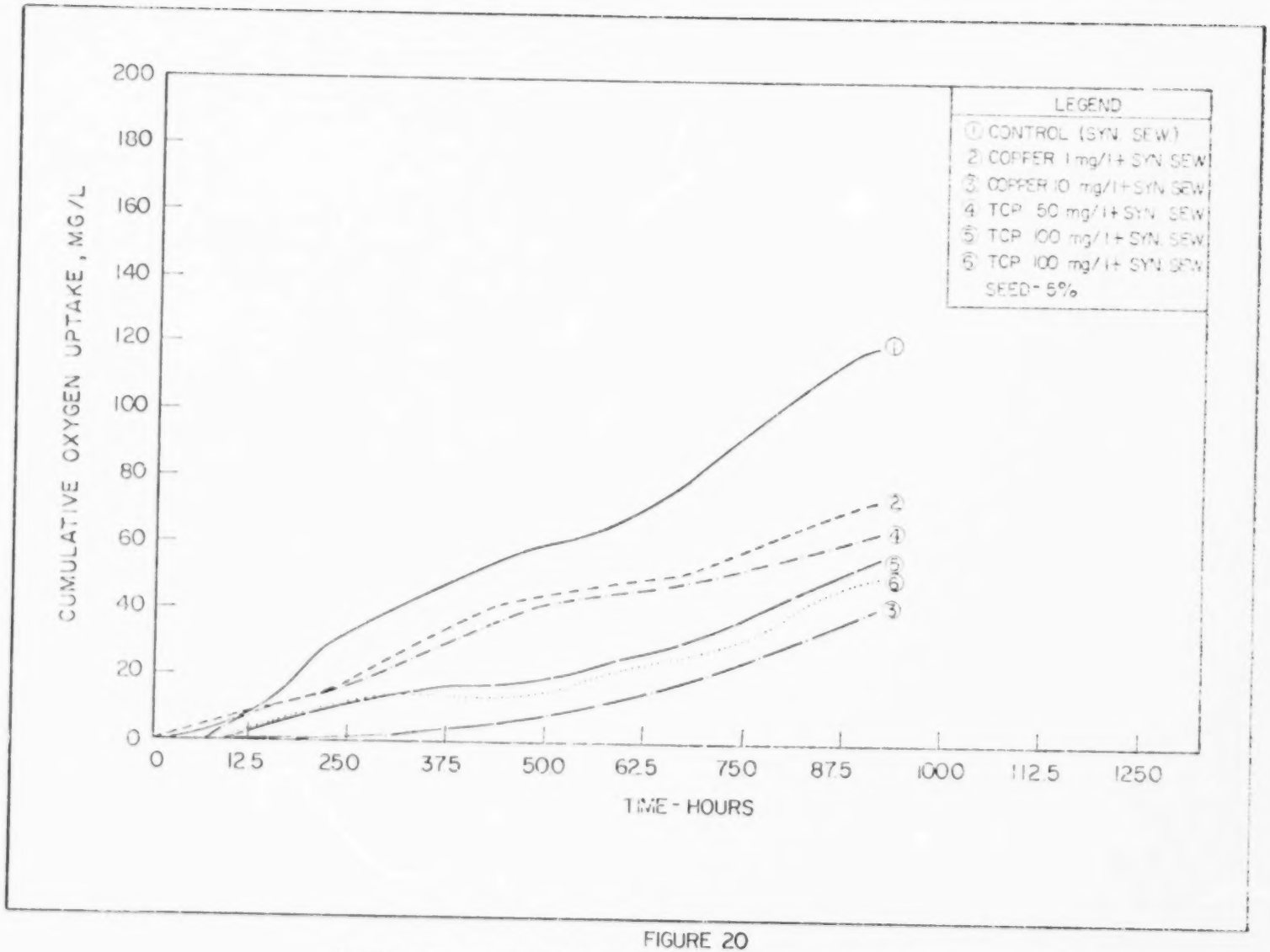


FIGURE 20
EFFECT OF 2,4,6-TRICHLOROPHENOL ON A MIXED MICROBIAL POPULATION

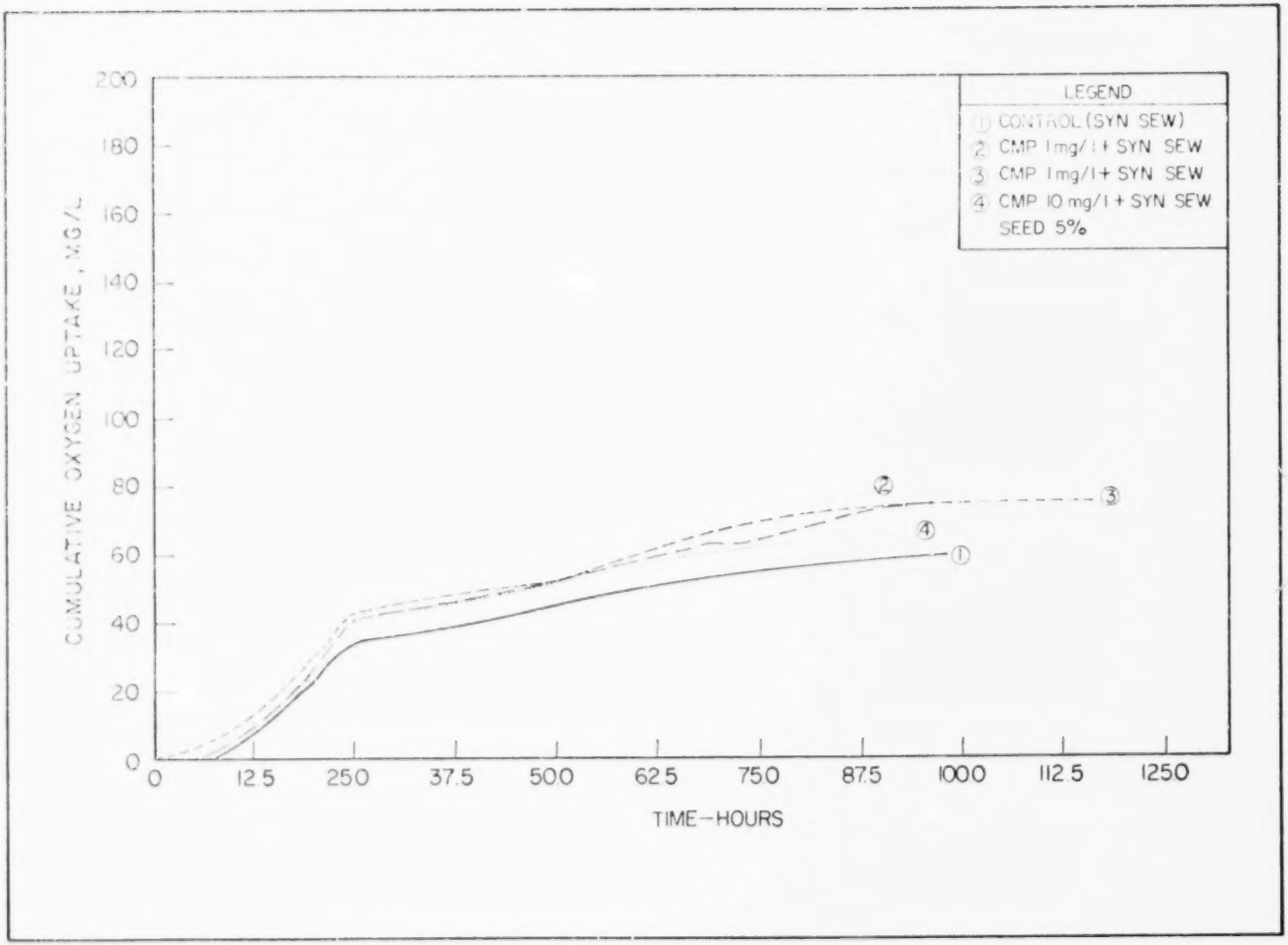


FIGURE 21

EFFECT OF 4-CHLORO-3-METHYLPHENOL ON A MIXED MICROBIAL POPULATION

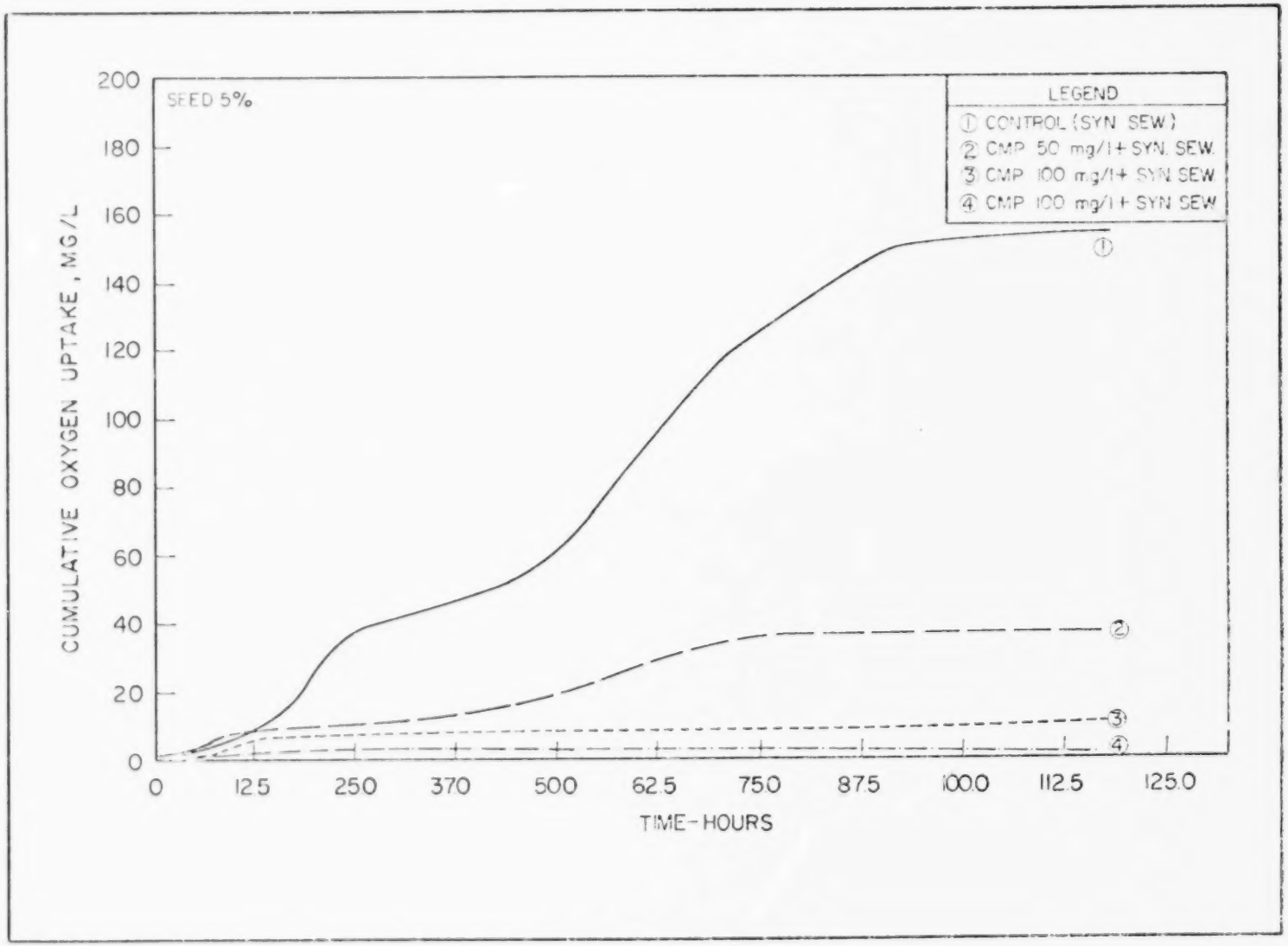


FIGURE 22

EFFECT OF 4-CHLORO-3-METHYLPHENOL ON A MIXED MICROBIAL POPULATION

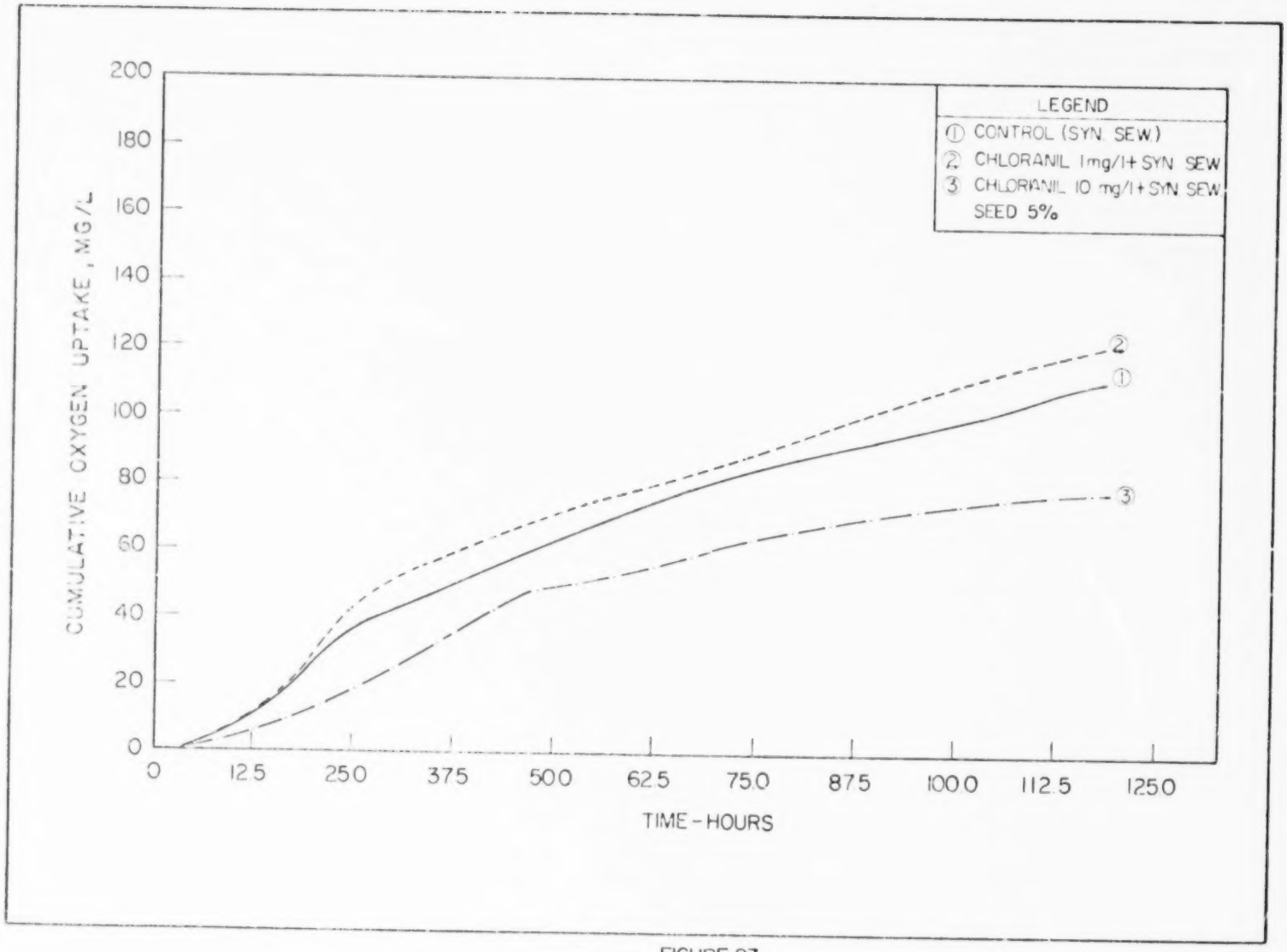


FIGURE 23
EFFECT OF CHLORANIL ON A MIXED MICROBIAL POPULATION

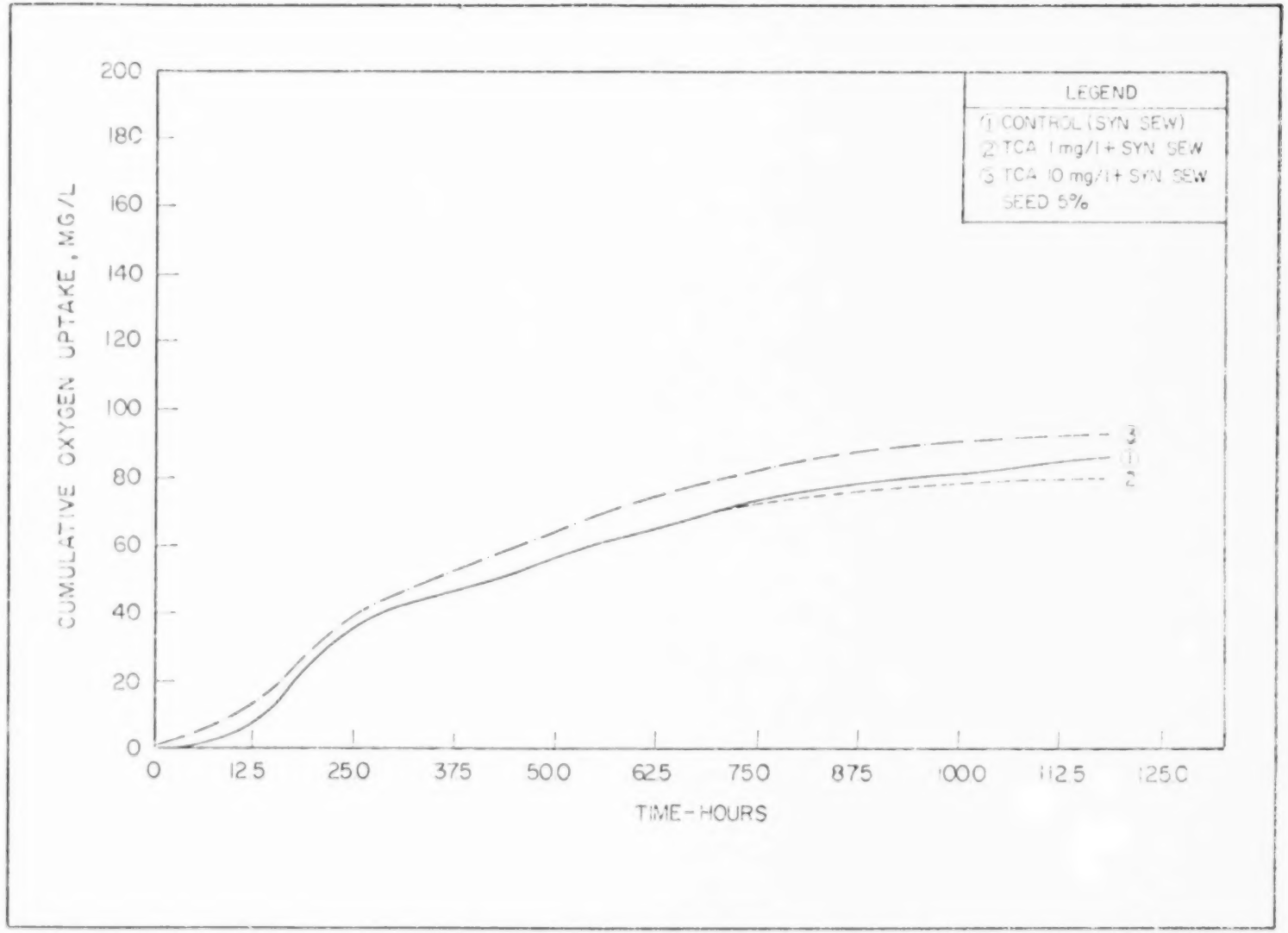


FIGURE 24
EFFECT OF 2,4,6-TRICHLOROANILINE ON A MIXED MICROBIAL POPULATION

degraded at a rate sufficiently high to make its persistence in an effluent improbable.

SECTION IX

STATIC BIOASSAYS – PHASE II

Routine static bioassays were conducted to determine the four-day median tolerance limits of an appropriate test organism to the test compounds. For purposes of this study, the TL_m was established to within one order of magnitude.

The four-day static bioassays were conducted according to Standard Methods[5] procedures.

Chlorine-free tap water which had been vigorously aerating for two days prior to the start of testing, was used as dilution water. Fathead minnows (Pimephales promelas) were used as test organisms. Three-gallon glass aquaria, each holding ten liters of water, were used as bioassay containers. Three separate series of static bioassays were conducted.

Series Number One

Four compounds were tested at three different concentrations each: 2,4,6–trichlorophenol (TCP) and 4–chloro–3–methyl phenol, (CMP), both at 100, 10, and 1 mg/l; 2,4,6–trichloroaniline (TCA), at 10, 1, and 0.1 mg/l; and chloranil at 1, 0.1, 0.01 mg/l.

The two phenolic compounds were added to the test containers as aqueous solutions. Two containers, each holding five test fish, received one concentration, bringing the total to six containers and thirty test fish for each of the two compounds. In addition, two containers, each holding five fish in straight diluent water, served as controls.

The other two compounds, 2,4,6–trichloroaniline and chloranil, because of their relative insolubility in water, were added to the test containers as methanol solutions. The facilities for testing each of the two compounds were exactly as described for the two phenolic compounds. Additional controls had to be set up to determine the effects of the methanol that was added with the compounds. Since the chloranil in methanol solution was of such strength as to result in a 400 mg/l methanol concentration in the test containers when the chloranil concentration was 1 mg/l, two control containers, each holding five fish in 400 ppm methanol in diluent, were provided. The 2,4,6–trichloroaniline in methanol solution resulted in an 80 mg/l methanol concentration when the TCA concentration was 10 mg/l. Therefore, two additional control containers were set up for 80 mg/l methanol in diluent.

Over the four-day period of testing, the percent mortality of all control fish was zero.

The 96-hour median tolerance limits of fathead minnows to each of the compounds tested were: 2,4,6-trichlorophenol <1.0 >.1 mg/l; 4-chloro-3-methyl phenol at <.1 >.01 mg/l; 2,4,6-trichloroaniline at <10 >1.0 mg/l; and chloranil at <1.0 >.1 mg/l.

The test results are presented in Figures 25 through 28. The chloranil test fish exhibited a somewhat unusual reaction pattern because of the insoluble nature of the compound. Despite precautions, the chloranil flaked out when the methanol solution mixed with the diluent water, and floated or sank to the container bottom. Some of the test fish were seen ingesting these solid particles, predominantly at the 1 mg/l concentration level, and exclusively at the start of testing. The remaining fish did not do the same, and consequently, survived the 4-day period. At the lower concentrations, only a few fish were affected. More fish died at the 0.01 mg/l concentration than at the 0.1 mg/l concentration, probably because more solid particles of chloranil were ingested by those fish.

Series Number Two

One compound, p-benzoquinone, was tested at three concentrations, 10, 0.5, and 0.1 mg/l. The compound was dispensed to the test containers as an aqueous solution. Exactly the same facilities, including controls, were used for benzoquinone as were used for each of the phenolic compounds in Series Number One.

All of the test fish died within the first day of testing over the entire concentration range, while the control fish survived. However, the control fish mortality was too great after 4 days to satisfy Standard Methods criteria for test validity. It was thought that perhaps the benzoquinone vaporized from some of the test containers and contaminated the control diluent waters. Consequently, a third bioassay was set up to restudy benzoquinone. In this study, the control containers were well separated from the test containers. Despite the unsatisfactory behavior of the control fish, this test did yield valid information. The reaction of benzoquinone was immediate and violent. It was decided, therefore, to test the compound at lower concentration levels in Bioassay Number Three, starting with 0.1 mg/l.

Series Number Three

Benzoquinone was tested at three concentrations, 0.1, 0.01, and 0.001 mg/l. The same procedures as used in Bioassay Number Two were followed, except that four fish per container were used. The results of Bioassay Number Three are presented in Figure 29.

All of the test fish in the 0.1 mg/l benzoquinone water died within the first two days of testing, while the lower concentration levels had no significant effect. However, the control

FIGURE 25

RESULTS OF STATIC BIOASSAY SERIES NO.1

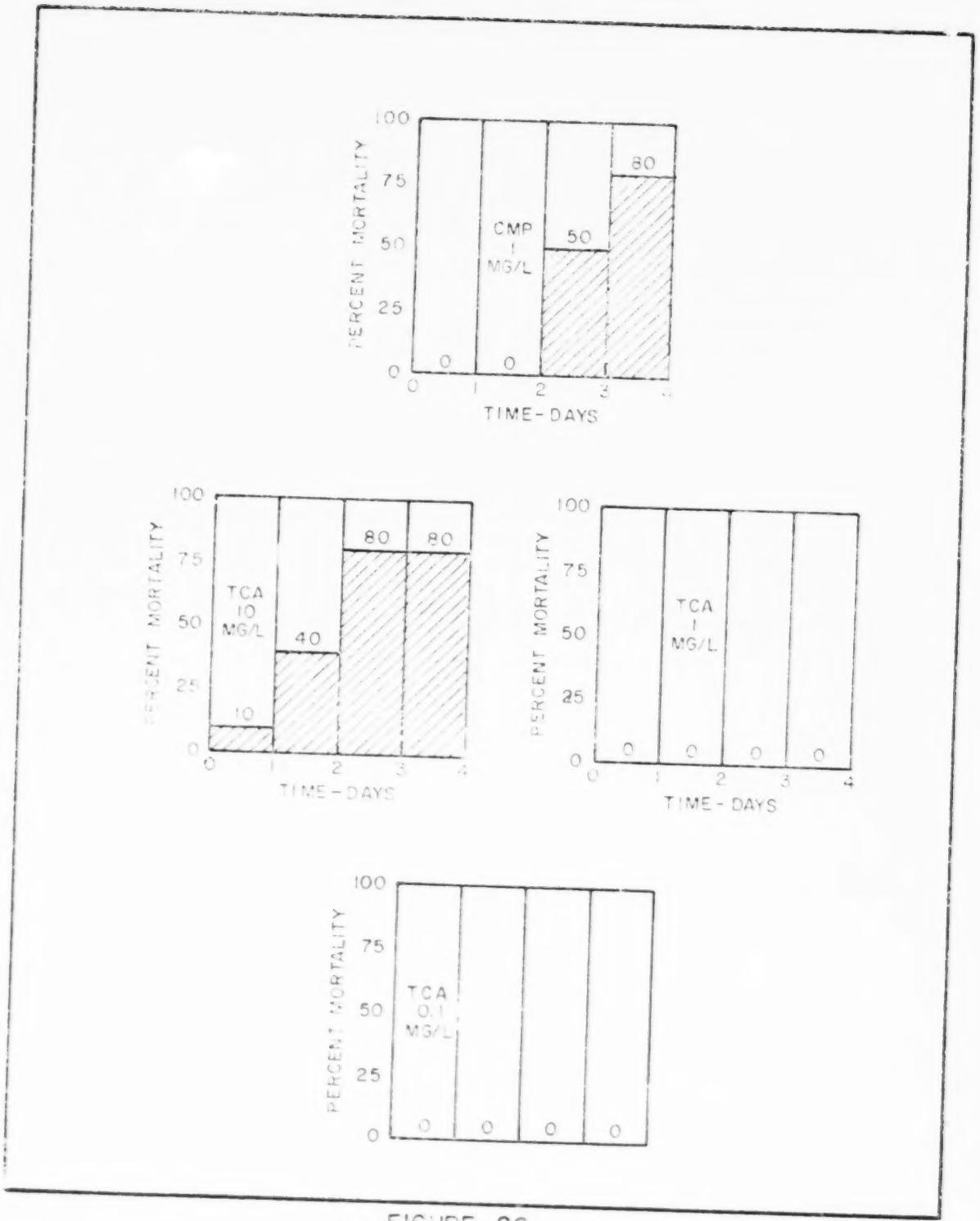


FIGURE 26

RESULTS OF STATIC BIOASSAY SERIES NO. 1
(CONTINUED)

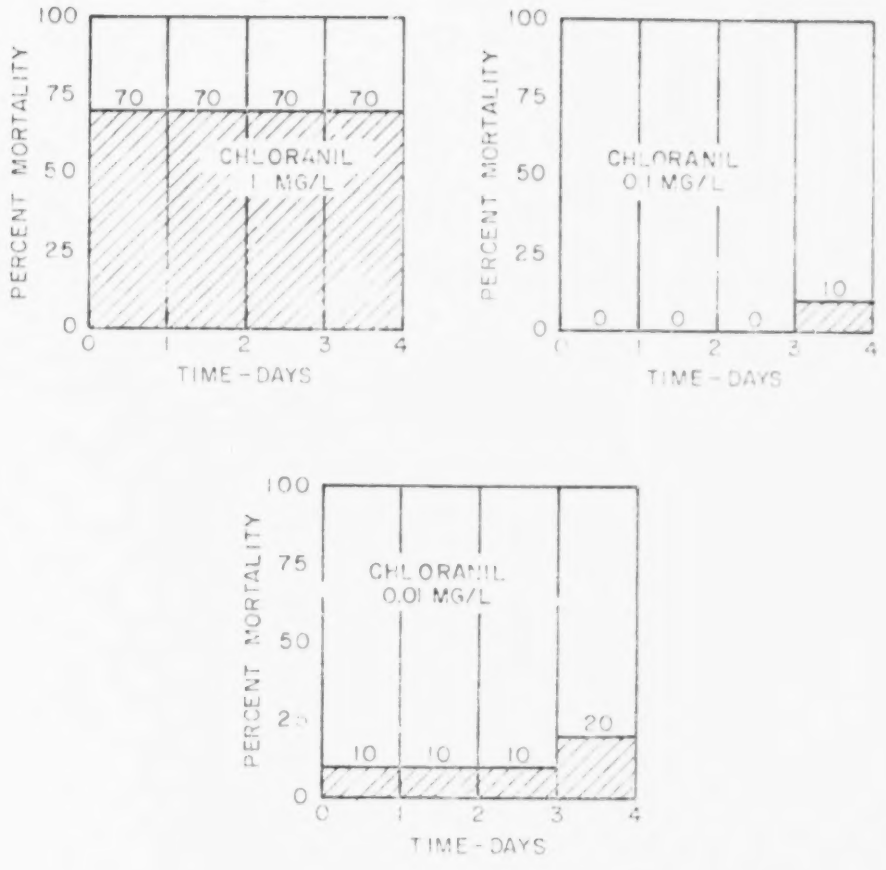
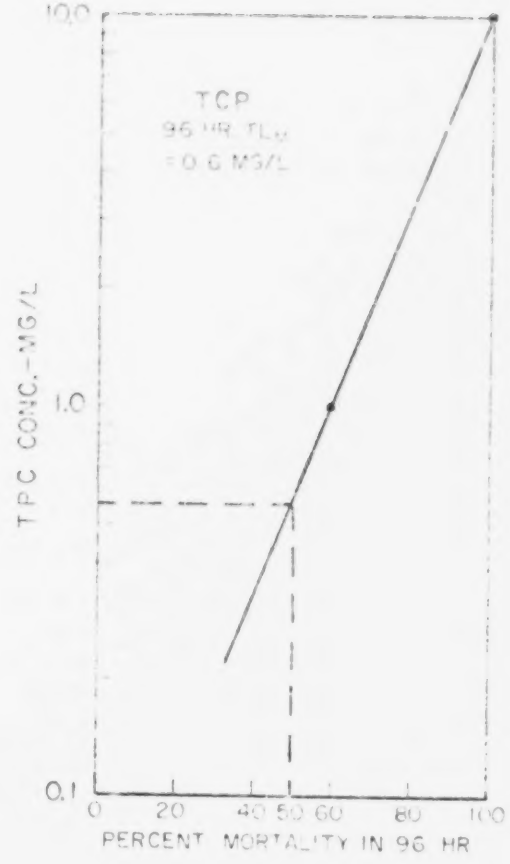


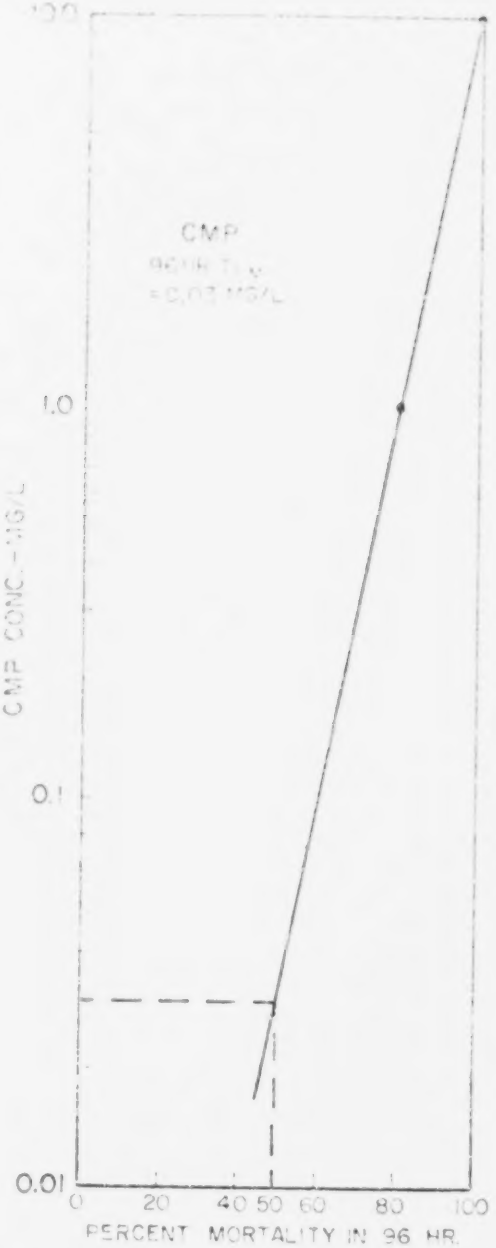
FIGURE 27

RESULTS OF STATIC BIOASSAY SERIES NO. 1
(CONTINUED)



A

4-DAY MEDIAN TOLERANCE LIMIT DETERMINATION FOR 2,4,6-TRICHLOROPHENOL



B

4-DAY MEDIAN TOLERANCE LIMIT DETERMINATION FOR 4-CHLORO-3-METHYL PHENOL

FIGURE 28

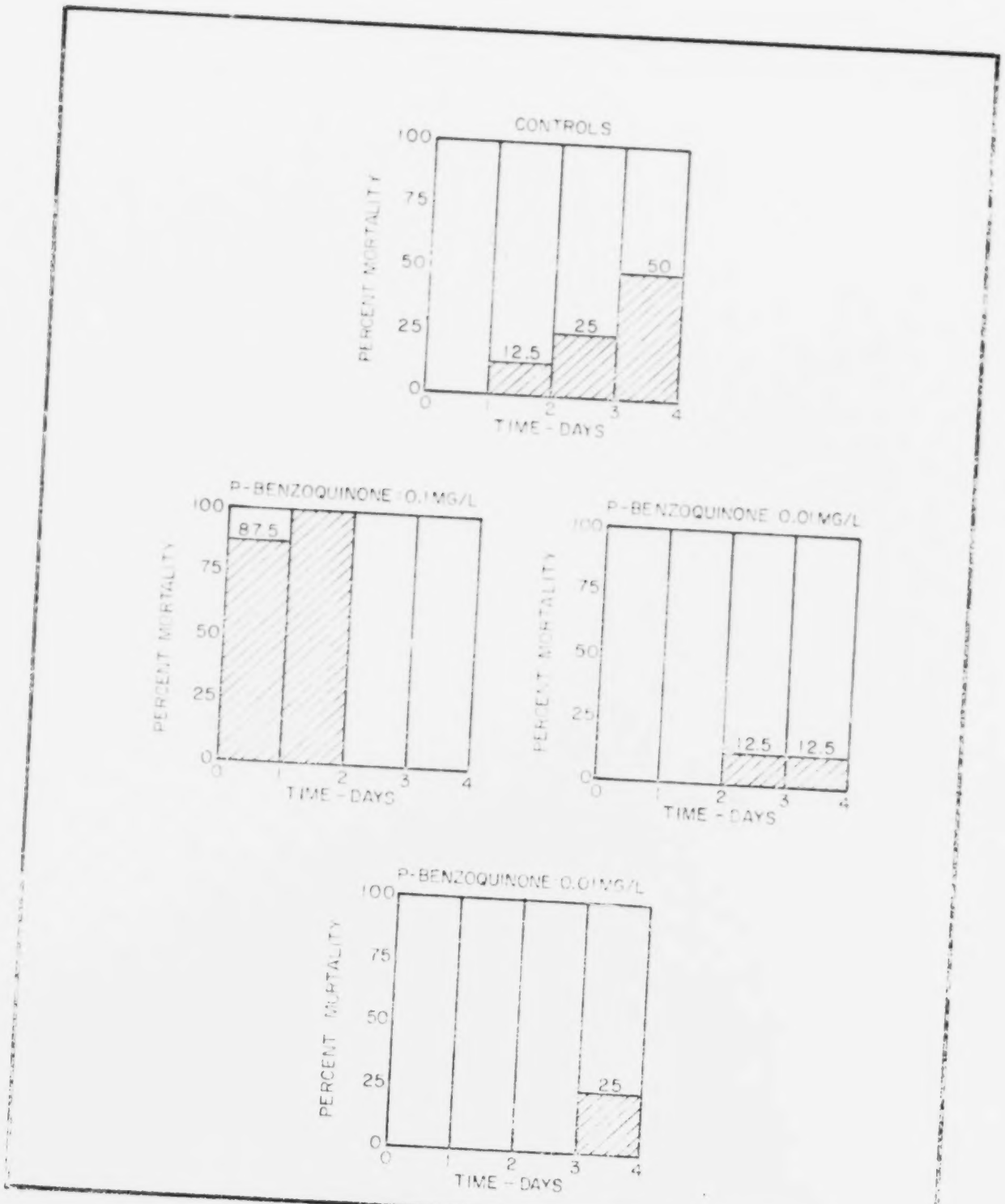


FIGURE 29

RESULTS OF BIOASSAY NO. 3 – P-BENZOQUINONE

fish again exhibited high mortality after 4 days and this test did not meet the Standard Methods criteria for validity either. However, control losses were not as drastic as in the previous bioassay, and the lower concentration levels of the compound seemed non-toxic. The 96-hour TL_m probably has a value of about 0.005 to 0.03 mg/l, as seen in Figure 29.

Summary

Five chlorinated organic compounds were tested for toxicity to fathead minnows. Of the five, four 96-hour median tolerance limits were determined. For the fifth compound, p-benzoquinone, a probable toxic concentration was determined. These values are presented in Table 14.

TABLE 14

RESULTS OF STATIC BIOASSAYS

Compound Tested	96-hour TL_m Range (mg/l)
2,4,6-trichlorophenol	1.0 - 0.1
2,4,6-trichloroaniline	10 - 1.0
4-chloro-3-methyl phenol	0.1 - .01
chloranil	1. - 01.

Compound Tested	Probable Toxic Concentration Level (mg/l)
p-benzoquinone	< 0.1

SECTION X

PHASE THREE

Selection of Study Compounds

As a consequence of study limitations, it was required to limit the Phase III studies to three compounds. 4-chloro-3-methyl phenol was eliminated in view of the experimental data indicating the greater probability of occurrence of the 2,4,6-trichlorophenol. Chloranil was eliminated in view of significant question as to whether the compound can actually be formed under treatment plant conditions. The substrates selected for Phase III study were:

2,4,6-trichlorophenol
2,4,6-trichloroaniline
p-benzoquinone

Flow Through Bioassay Studies

Static bioassay investigations, such as performed in Phase II of the study have long been undertaken to evaluate toxicities of test substrates. The ease of performance and low cost of such studies are attractive, but many questions have arisen concerning the translation of such results to prototype conditions. Ideally, a broader study considering many aspects of the food chain over a longer period of time would be desirable. The cost and facilities required for conducting large scale bioassays has sharply curtailed investigations of this nature. This project chose to consider an intermediate scale bioassay, flow through ecosystem that could be operated at a reasonable cost, but would provide better information on environmental response at several trophic levels.

Fish, vascular plants, macroinvertebrates, and microscopic plants and animals were investigated. Twenty gallon aquaria with flow through water systems were chosen as the study systems, and study periods of approximately one month per study conduction were considered.

SECTION XI

RESULTS OF PHASE III

Fish

Fathead minnows obtained from a local hatchery were employed as a test organism. Die-off of the test fish occurred in most of the experimental units during the background acclimation periods and in the control units during the bioassays, complicating data interpretation.

Figure 30 is a graphic representation of the number of test fish surviving versus time, for each experimental unit. In Table 15, the duration of the study is broken down into convenient periods and the percent mortality is given for each period and for the whole study.

TABLE 15

TABULAR SUMMARY OF FATHEAD MINNOW MORTALITIES

Control Units

Unit #	Percent Mortality*				
	Oct. - Feb.	Mar.	April	May - June	Total
1	6	0	25	10	37
8	6	0	18	0	23

Units Subjected to Addition of Compounds

Unit #	Percent Mortality*				
	Background	Conc. #1	Conc. #2	Conc. #3	Total
Benzo.					
4	6	4	11	100	100
5	13	19	24	100	100
TCP					
6	0	30	14	100	100
7	17	0	24	100	100
TCA					
2	10	22	0		30
3	20	8	0		27

*percent of the remaining population entering a particular stage (Concentration #1, etc.,) that died during that stage.

The control fish populations were stable from the beginning of the study until the middle of April. From this time until the beginning of May, a high rate of die-off occurred, reaching 25% in one case. This can only be attributed to disease, or some natural cause, since no contamination by any of the compounds was shown in spectrophotometric analyses.

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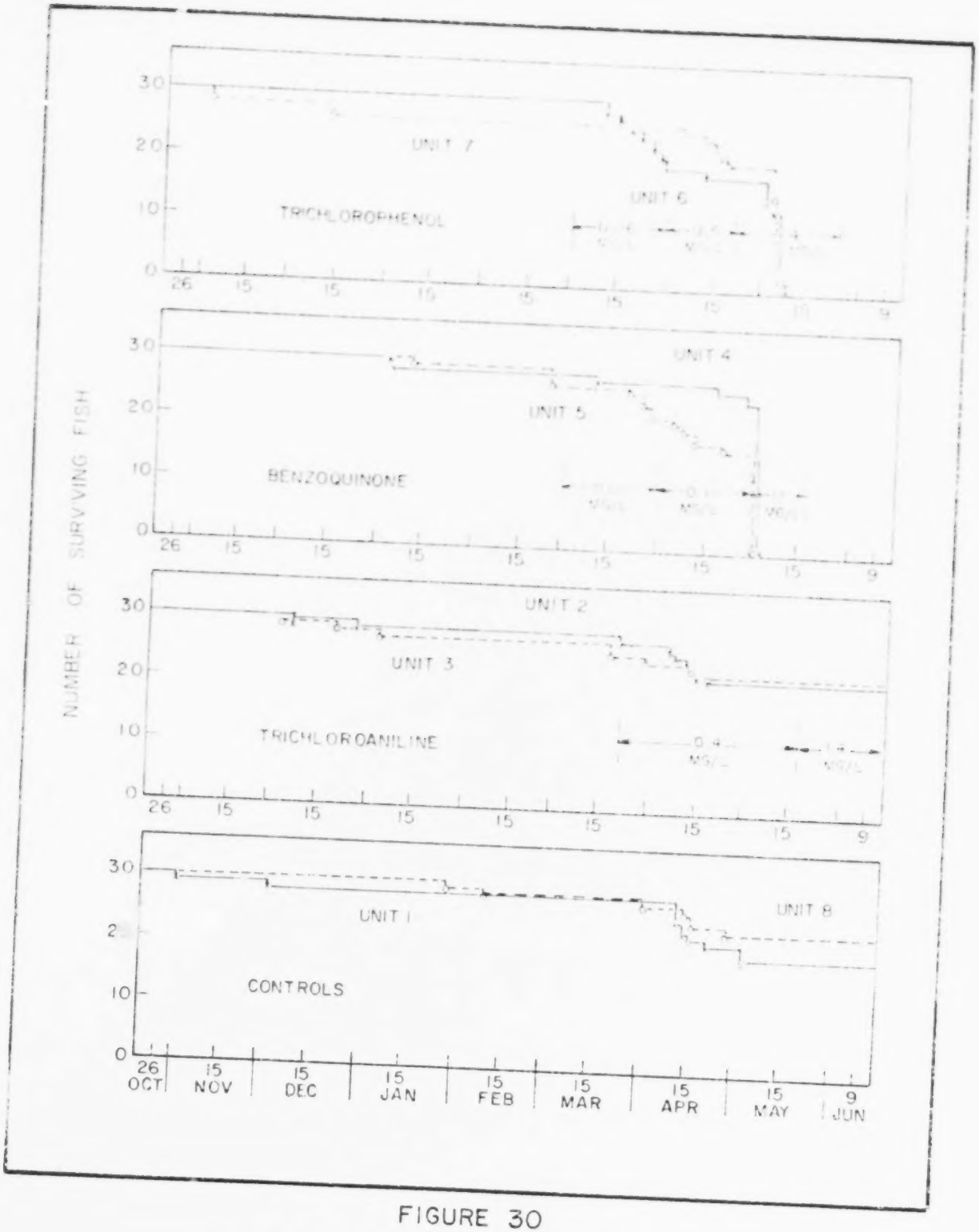

FIGURE 30

FATHEAD MINNOW POPULATIONS VS. TIME

The total percent mortality of the control fish through the entire duration of the experiment varied from 23% to 37%. Therefore, no significant toxic effect may be attached to lower mortalities in any of the test units.

TCA showed no effects on the test fish at any concentration level. This is consistent with the findings of the four-day static bioassay, since the 96-hour TL_m as determined by that test was not exceeded or even reached in the flow-through studies.

Benzoquinone and TCP exhibited no significant effect at either of the two lower concentration levels. Even at the second concentrations, which compared with the 96-hour TL_m's, the mortality was less in these test units than in the controls. This would seem to indicate either a greater resistance of the test fish to both of these compounds when in a more natural environment or the degradation or assimilation of the compounds within the aquaria. Spectrophotometric measurements indicated that some reduction in concentration did take place in the units. A comparison between applied concentrations and observed concentrations is presented in Table 16.

TABLE 16

CONCENTRATIONS (mg/l) OF SUBSTRATES DURING VARIOUS TEST PERIODS

Substrate	Period 1		2		3	
	Applied	Measured	Applied	Measured	Applied	Measured
p-benzoquinone	0.01	(1)	0.108	0.006	1.15	0.23
2,4,6-trichloroaniline (TCA)	-	-	0.4	0.14	0.135	NA
2,4,6-trichlorophenol (TCP)	0.06	(1)	0.47	0.25	4.15	1.75

(1) below measureable range.

When the third concentration levels of benzoquinone and TCP, values ten times greater than their 96-hour TL_m's, were dispensed, the reaction of the test fish was immediate and violent. Within a few days after starting these bioassays, 100% kill was effected in all four test units. Compound measurements indicated that these lethal concentrations were somewhat less than half of the nominal levels delivered to the aquaria.

Vascular Plants

The growth of two vascular plants, Ludvigia and Anacharis, was studied in detail. The total length of all Ludvigia stalks in each test aquarium was 35 to 40 inches at the beginning of the study, and had increased to 75 to 100 inches by the middle of May for the control, benzoquinone, and TCP units. The plants in the TCA units had grown to over 100 inches by the end of May. None of the test compounds affected the health and growth of Ludvigia at any concentration level.

The growth rates of Anacharis varied so extremely among the aquaria during the background period of the study that comparability of the units to one another was severely hampered. As stated in Table 17, the average growth rates varied from less than one inch per week to more than seven inches per week. Prior to the start of the bioassays, the Anacharis plants were re-distributed among the eight units so that each contained approximately the same number of inches of total stalk length.

TABLE 17

VASCULAR PLANTS GROWTH

Unit	Average growth rate in inches per week* over background period		Average growth rate in inches per week* over bioassays period+	
	Ludvigia	Anacharis	Ludvigia	Anacharis
Control unit 1	1.1	0.9	3.5	2.4
Unit 8	0.9	1.7	3.8	5.7
Benzoquinone				
Unit 4	1.0	7.1	4.9	1.8
Unit 5	0.9	4.5	3.0	3.0
TCP				
Unit 6	0.8	3.2	3.4	5.4
Unit 7	0.8	5.6	4.1	6.3
TCA				
Unit 2	1.6	3.2	5.7	6.9
Unit 3	1.3	4.3	4.7	5.1

*That is, the total number of inches of new growth contributed by all six main stalks.
†From beginning of Conc. 1 to end of last Conc.

During the bioassays, the average growth rates of Anacharis in the benzoquinone test aquaria were the slowest, showing a decrease from the background rates. The growth rates increased in all otner units. However, the control plants in Unit #1

still exhibited very slow growth, less in fact than benzoquinone Unit #5. Also, benzoquinone Unit #4 suffered from the redistribution of plants, since it was given some of the slowest growing stalks from Unit #1.

In summary, the inconsistancy of Anacharis growth proved to be too great to use this plant as an indicator of compound effects. It was observed, however, that none of the compounds affected the general appearance of outward health of Anacharis.

Benthic Macroinvertebrates

The macroinvertebrate populations underwent so sharp a decrease over the course of the background acclimation period as to render them unsuitable as test organisms. As shown in Figure 31, out of 93 individuals (excluding Tubifex) present in each aquarium at the time of initial seeding, an average of only 15 per aquarium could be observed in mid-March, a decrease of [illegible]%. The average Hydropsychidae population decreased 90%, fr[illegible] 31 to 3 individuals; Asellus decreased 77%, from 13 to 3; E[illegible]opria 69%, from 13 to 4; Gyraulus 79%, from 14 to 3; Helobdella 88%, from 17 to 2; and Ferrissia 100%.

Several reasons for this drastic decline can be identified. Some of the organisms avoid observation, either by burrowing into the benthic sediments when observations were made, or by burrowing into the sand matrix. Some of the organisms are consumed by the test fish. The fish were not the only predatory stress on the macroinvertebrates. Asellus is a potential predator on all of the other animals, and Helobdella preys upon Gyraulus and Ferrissia. Since no effort was made to separate the various types of macroinverbrates from one another, Helobdella and Asellus may have consumed some of the other organisms.

Adequate food supplies may have been lacking. Specifically, the microflora and fauna populations may have been too low to support the macroinvertebrates during the background acclimation period. The lack of nutrients in the diluent water may have too severly limited the rapid growth of algae necessary for a productive ecosystem.

Fish waste products and other detritus built up in the aquaria with time, settling on the benthos in thickening sludge layers. Perhaps the gases of decomposition released through bacterial decomposition of this waste proved injurious to the macroinvertebrates. This bacterial activity also may have depleted the dissolved oxygen resource at the benthos-water interface.

Finally, the marked drop in the caddis fly larva population was influenced by a special factor unique to Hydropsychidae, its annual metamorphosis from larva to pupa to adult. Only the larva may be used as a test organism, since it is active in

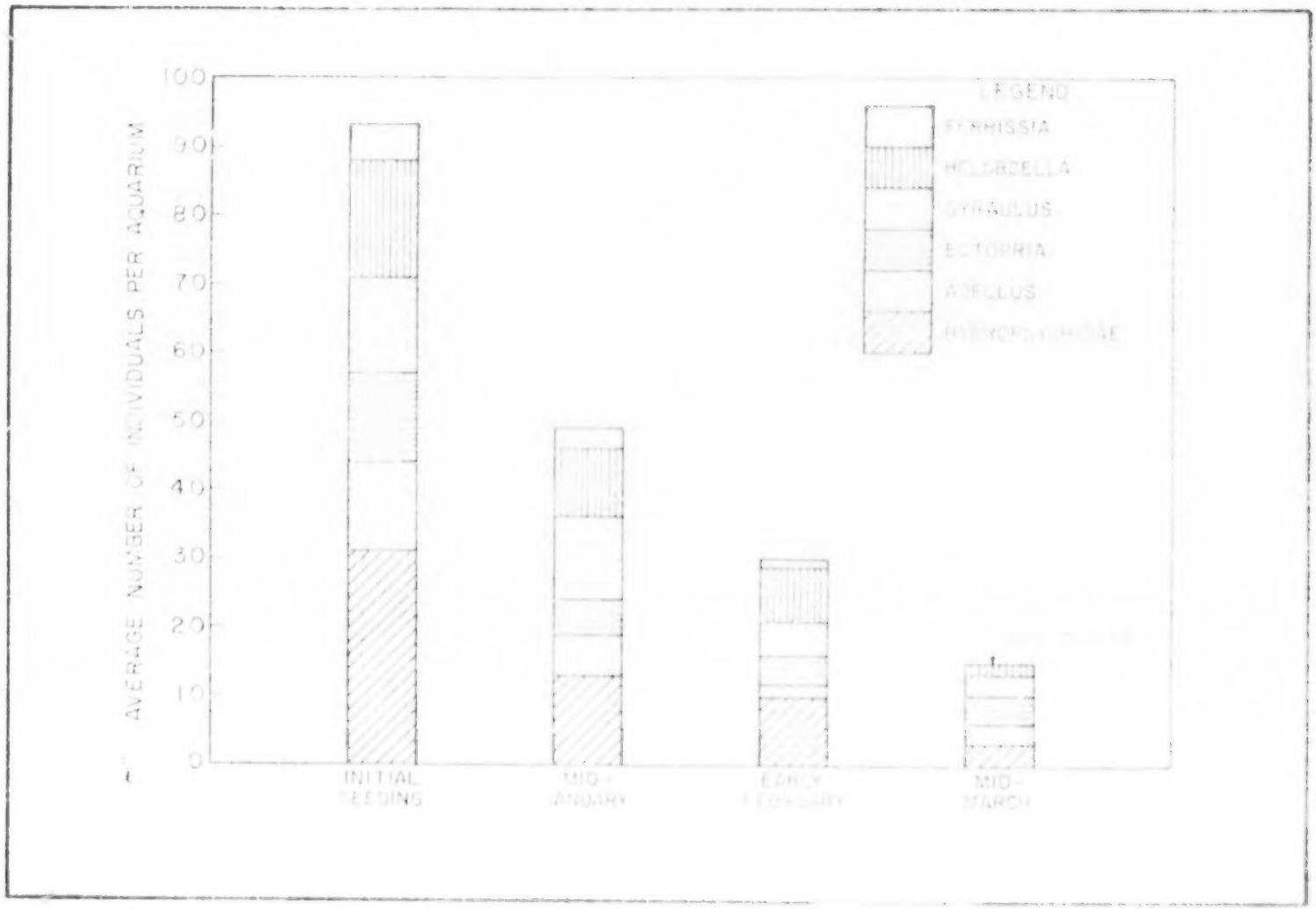

FIGURE 31

BACKGROUND MACROINVERTEBRATE POPULATIONS VERSUS TIME

water, while the pupa is immobile within its cocoon and the adult is a terrestrial organism. In several instances from February to March, immobile cocoons and adults struggling to leave the water were observed. A significant portion of the Hydropsychidae population may have undergone metamorphosis at this time.

Microscopic Flora and Fauna

A total of six sets of population counts were made, three of two-week populations, and three of four-week populations. The significance of each of these sets is listed below:

Four-week set No.1. – compares control populations of April 5 to benzoquinone and trichlorophenol populations developing at the end of the first concentration levels of these compounds;

Four-week set No.2 – controls on May 5; benzoquinone and TCP at the end of the second concentration levels; and TCA near the end of the first concentration level.

Four-week set No.3 – controls on June 1; benzoquinone and TCP approximately one week after the third concentration levels ended; and TCA about mid-way through the second concentration level;

Two-week set No.1 – controls on April 20; benzoquinone and TCP mid-way through the second concentration levels; and TCA about mid-way through the first concentration level.

Two-week set No.2 – controls on May 20; benzoquinone and TCP near the end of the third concentration levels; and TCA at the beginning of the second concentration level.

Two-week set No.3 – controls on June 15; and TCA just after the second concentration level ended.

The data from the microscopic observations program are summarized in Figures 32 through 38. For each pair of parallel experimental units, four population counts were made during each set of observations. The maximum, average, and minimum populations of each set were recorded.

Figures 32 and 33 are bargraphs of the average microfauna populations in each set of four-week and two-week observations, respectively, and each population is broken down into its components. Although the control populations exhibited considerable

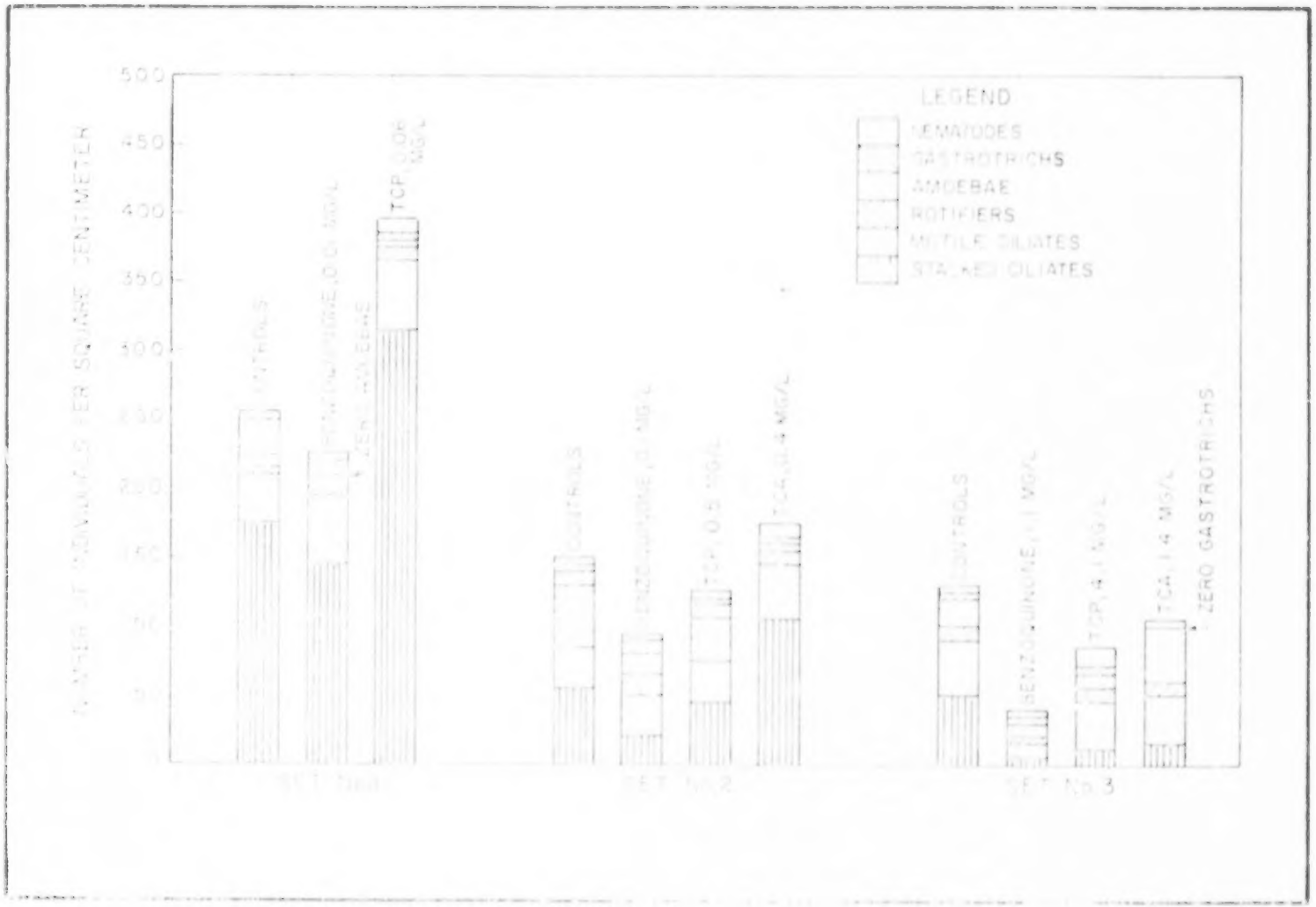

FIGURE 32

MAKE-UP OF AVERAGE 3-WEEK MICROFAUNA POPULATIONS

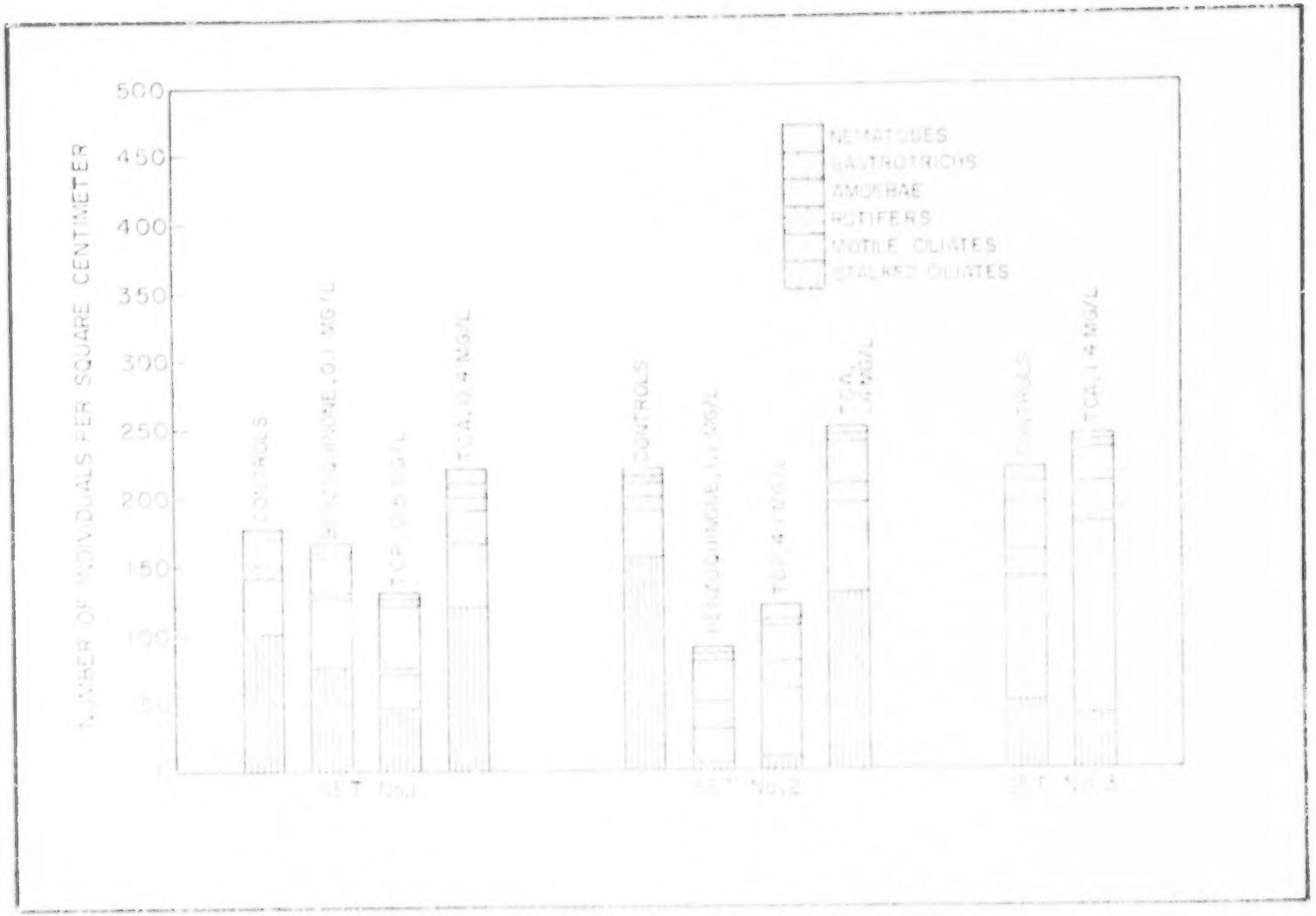

FIGURE 33

MAKE-UP OF AVERAGE 2 WEEK MICROFAUNA POPULATIONS

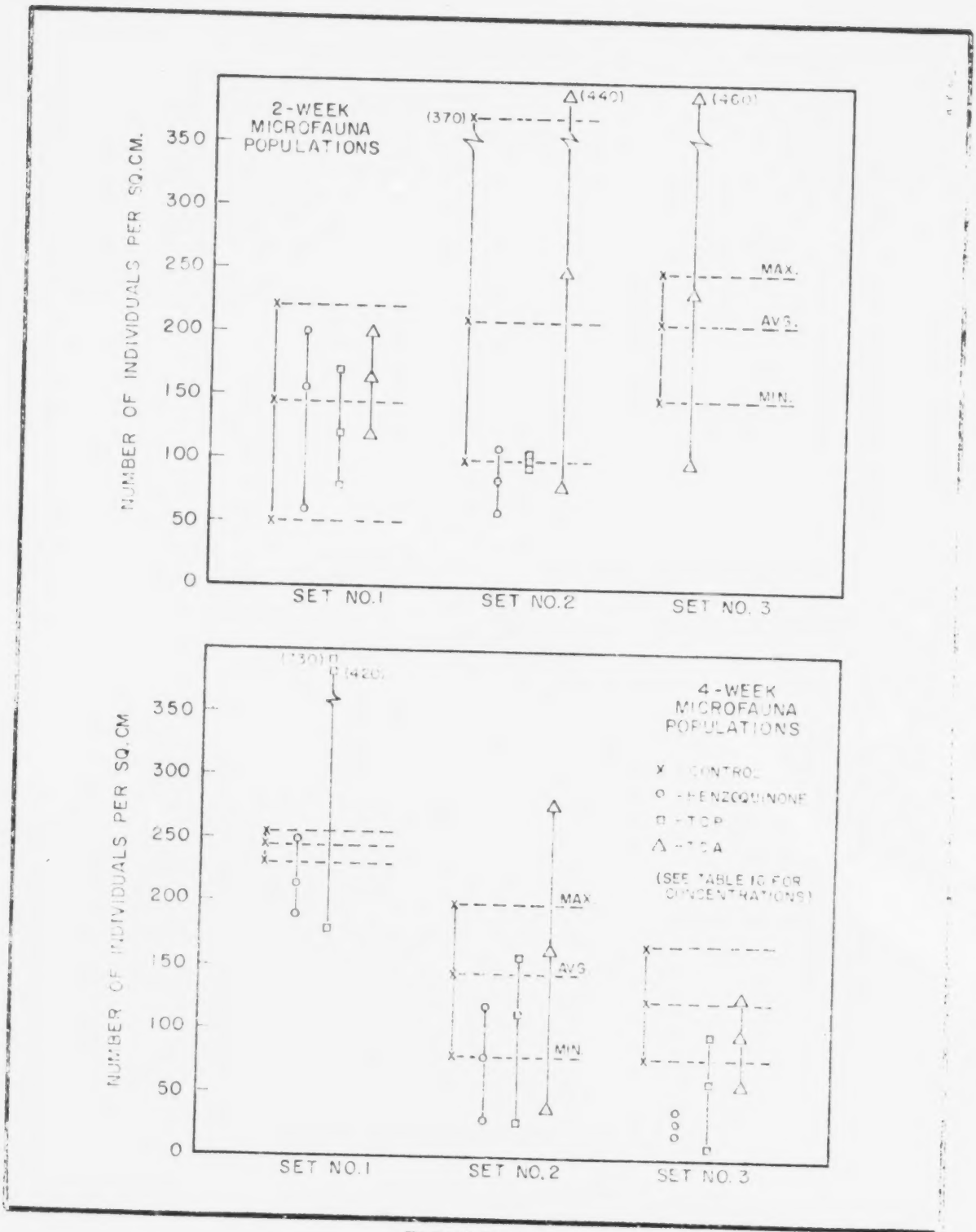

FIGURE 34

TOTAL MICROFAUNA POPULATIONS

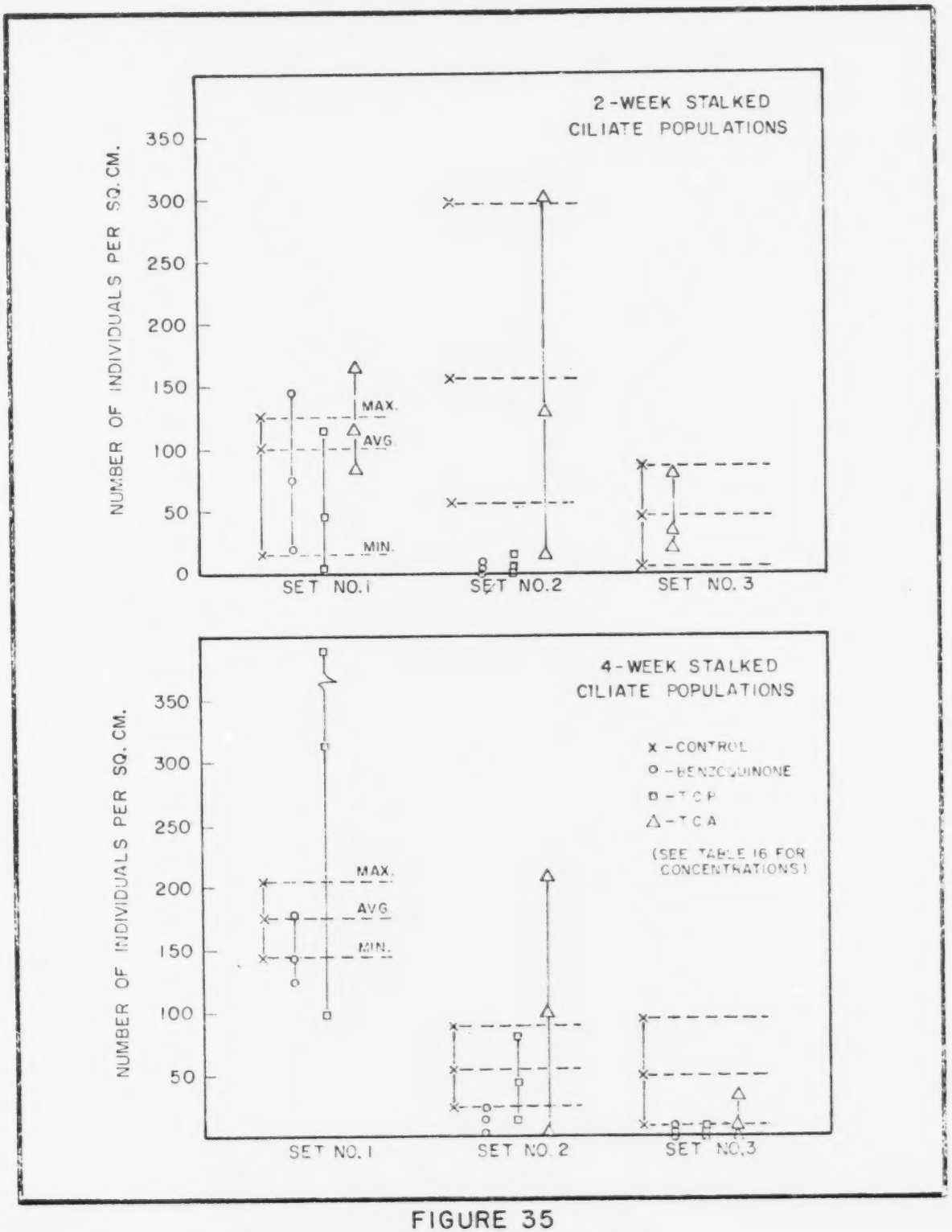

FIGURE 35

STALKED CILIATE POPULATIONS

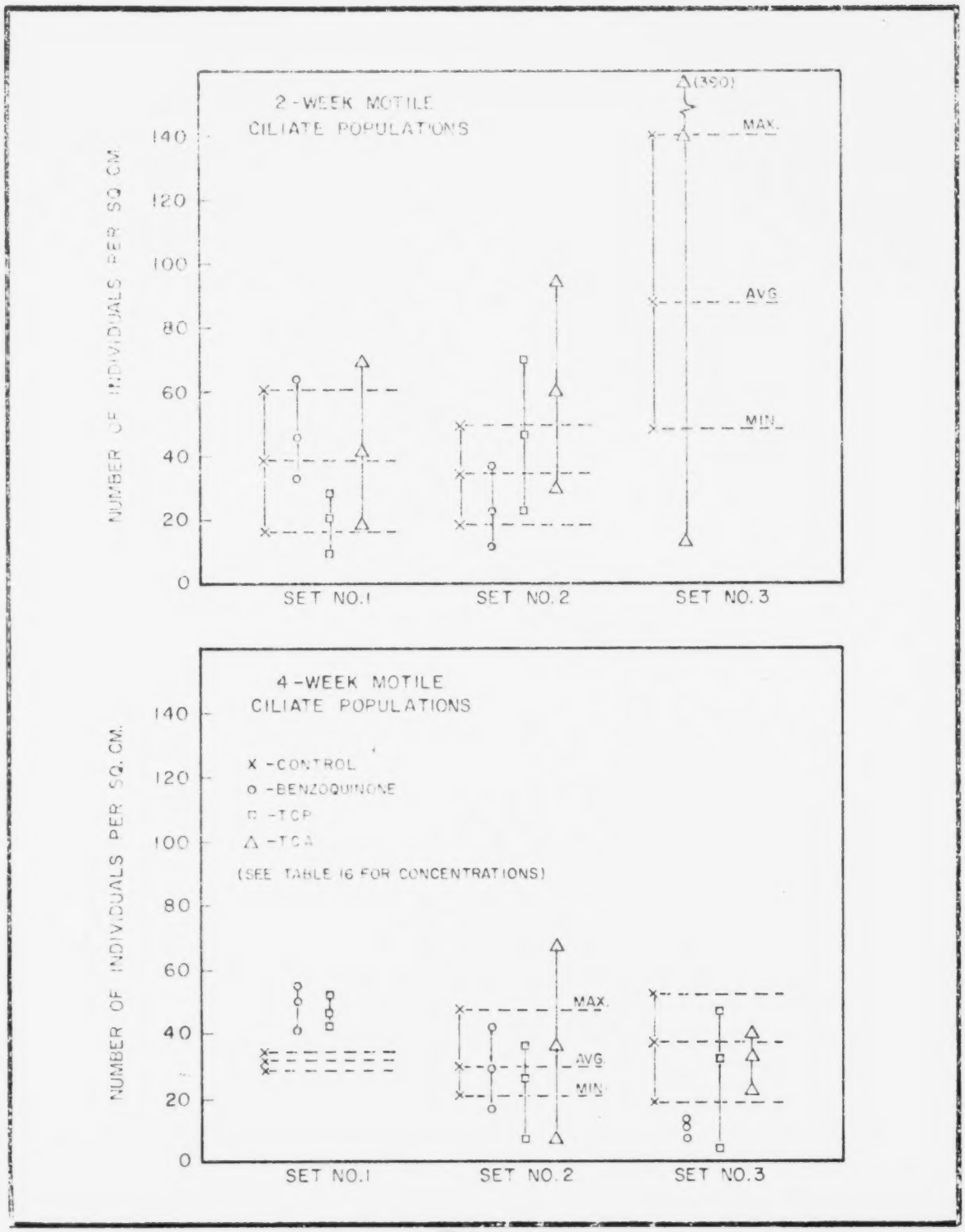

FIGURE 36

MOTILE CILIATE POPULATIONS

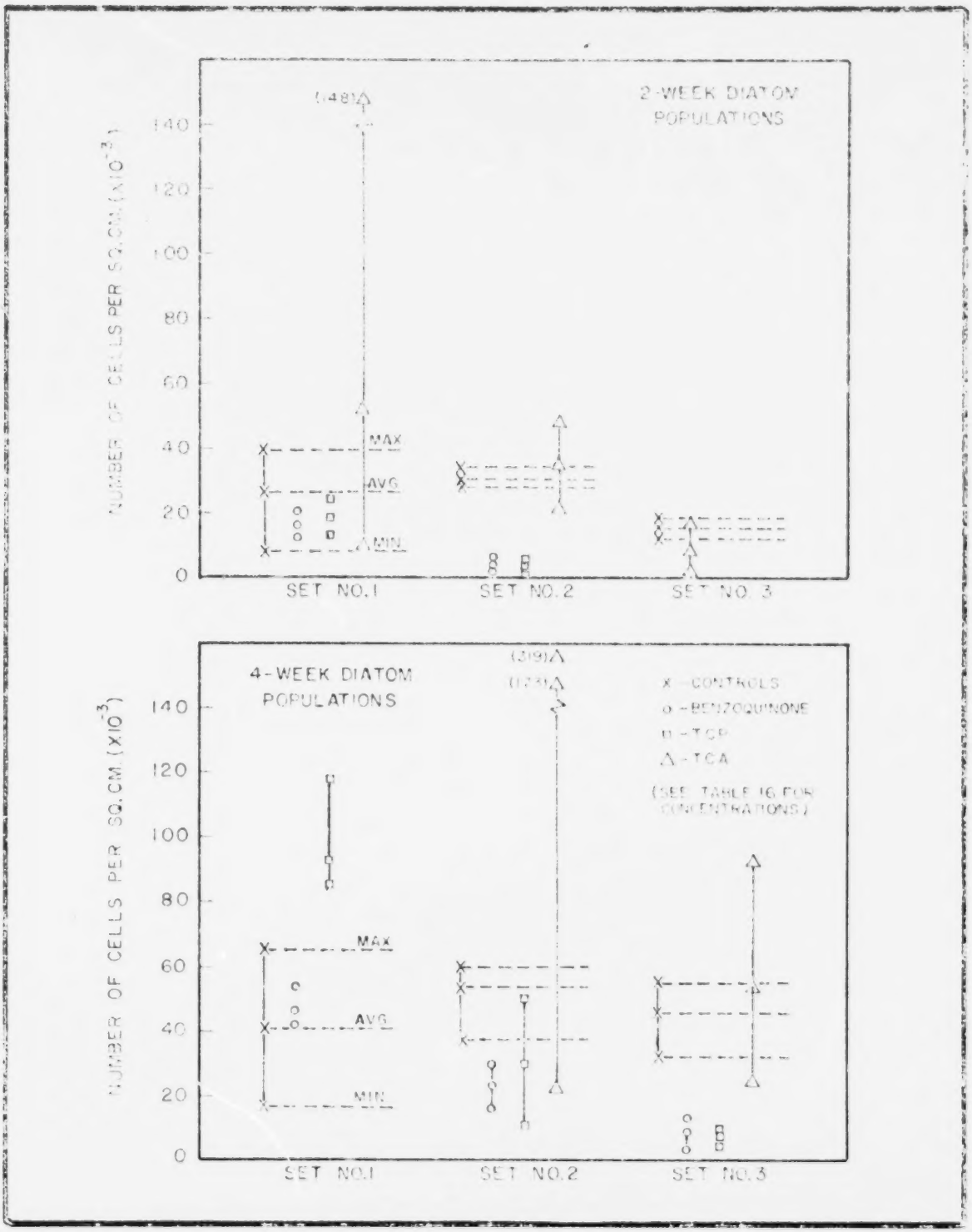

FIGURE 37

DIATOM POPULATIONS

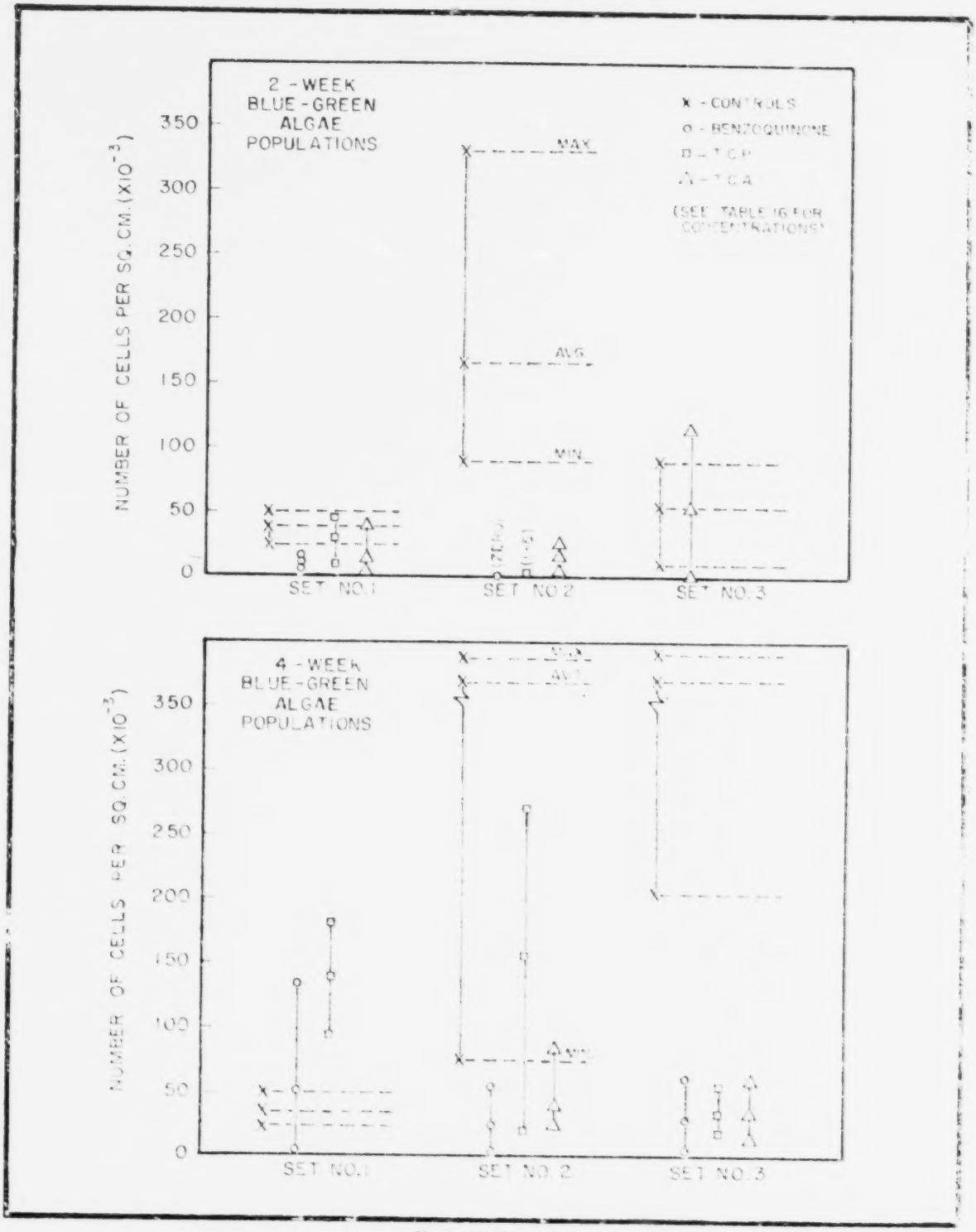

FIGURE 33

BLUE-GREEN ALGAE POPULATIONS

variation from one set to another, certain significant deviations occurred in some of the test populations. Most notably, both the four-week and the two-week benzoquinone populations show a decline at the end of the second concentration level, but especially during and after the third level, due primarily to the decrease in stalked ciliates. The average four-week control stalked ciliate population reaches a minimum of 50 individuals per square centimeter or 37% of the microfauna population, while the benzoquinone population drops to 5 individuals or 12% in observation set No.3. In a similar fashion, the average two-week benzoquinone stalked ciliate population drops to 5 individuals or 5% in observation set No.2, while the control minimum is 50 individuals, or 23%. It would seem then, that benzoquinone has an inhibitory effect on this organism at a concentration level of 1 mg/l (concentration No. 3) and possibly at a concentration as low as 0.10 mg/l (concentration No.2).

While the decline in the TCP total microfauna population is not as marked, the TCP stalked ciliate population drops to a minimum of 10 individuals in four-week observation set No.3 and two-week observation set No.2, being 12% and 8% of those microfauna populations, respectively. Thus, TCP seems to affect this organism at a concentration of 4 ppm (concentration No.3).

The TCA microfauna populations compare favorably to the controls although the four-week stalked ciliate population does decline in set No.3 to 15 individuals or 14%, indicating a possible inhibitory effect at the second concentration level (1.4 ppm).

In contrast to the stalked ciliates, the control motile ciliate population is quite stable over the course of the bioassays. Only one instance of significant deviation in the test motile ciliate populations occurs. The four-week benzoquinone population declines to a minimum average of 10 individuals per square centimeter in observation set No.3, as compared to a control minimum average of 30 individuals, indicating a possible inhibitory effect on this organism of the third concentration level.

The rotifer, amoeba, gastrotrich, and nematode populations fluctuated to such a great degree and were so small in comparison to the ciliate populations, as to make evaluation of possible compound effects insignificant.

Figures 34, 35, and 36 plot the maximum, average, and minimum populations of microfauna, stalked ciliates, and motile ciliates, respectively, for all observation sets. Note the decline in microfauna and stalked ciliate populations in the TCP and benzoquinone test units, and the general stability of the motile ciliate populations except for benzoquinone in four-week observation set No.3. The data on microfauna populations would

seem to indicate inhibitory effects of the compounds, especially with regard to stalked ciliates, at the following nominal concentration levels:

> benzoquinone at 1 ppm (concentration 3) and possibly 1/10 ppm (concentration 2); TCP at 4 ppm (concentration 3); and TCA at 1.4 ppm (concentration 2).

There appeared to be no effects on the microfauna of any of the compounds at lower concentration levels.

Four categories of microflora were observed in the experimental aquaria: diatoms, blue-green algae, green algae, and yellow-brown algae. Possible inhibitory effects were seen on two of these, namely, the diatom populations and the blue-green algae populations.

Figure 37 plots the maximum, average, and minimum diatom populations in all observation sets. The four-week control populations were exceptionally stable throughout the duration of the bioassays, ranging from 17,000 to 65,000 cells per square centimeter with an average of 47,000. The four-week benzoquinone populations exhibited a steady decline, finally deviating significantly in observation set No.3, with an average of only 9,000 cells and a maximum of 13,000. TCP exhibits a similar trend, declining to an average of 8,000 and a maximum of 10,000. TCA diatom populations remained somewhat higher than the control populations in both sets of four-week data.

The two-week control diatom populations were also stable, with a maximum of 40,000, a minimum of 9,000, and an average of 25,000 cells per square centimeter. In observation set No.2, both the benzoquinone and the TCP diatom populations averaged only 5,000 cells with maximum of 7,000. The TCA populations were again somewhat higher than the controls, except in set No.3, with an average and a maximum of 9,000 and 18,000, respectively.

Thus, there is inconclusive evidence of inhibitory effects on diatoms of benzoquinone at 1 ppm (concentration 3), and possibly 1/10 ppm (concentration 2), TCP at 4 ppm (concentration 3), and TCA at 1.4 ppm (concentration 2).

The control populations of blue-green algae, as evident in Figure 38, were extremely variable, reaching a maximum of more than one million cells and a minimum of 25,000 cells in four-week populations. The most significant observation lies in the fact that, while tremendous blue-green algal blooms occurred in the controls, in general the populations remained low in the test units. It is not known, however, if this was due to the action of the compounds, or to some physical vagary in the controls at the time of the blooms.

In summary, the data from the microscopic observation program indicate that all three compounds, especially benzoquinone and TCP, may possibly inhibit diatoms and stalked ciliates at the highest concentration levels of the compounds. Additional evidence suggests that benzoquinone may inhibit these microorganisms at its second concentration level. The variations in basic population in the control unit were significant, and a detailed statistical examination of the population variance would be necessary to quantitatively define the impact of the various test compounds. To carry out such a study would require the collection of a large number of observations, and would be beyond economic practicality.

Summary

Substantial insight into the validity of the intermediate-term, flow-through bioassay as a method for determining toxicity was gained. Laboratory facilities incorporating model freshwater ecosystems in a flow-through system were designed. Methods for testing compounds and monitoring the ecosystems' response to compounds were developed. Three chlorinated organic compounds were investigated for toxicity to a variety of aquatic test organisms.

It was found that the test fish, pimephales promelas, was able to tolerate higher concentrations of p-benzoquinone and 2,4,6-trichlorophenol in the flow-through studies than in the four-day static bioassays. As a result of its limited solubility in water, 2,4,6-trichloroaniline could not be dispensed to the model ecosystems at a concentration level as high as that determined to be lethal in the static tests. The variation in control fish populations during the flow-through bioassays made it impossible to determine sub-lethal effects of the compounds.

Qualitative analysis of microorganism population data suggested inhibitory or toxic effects on diatoms and stalked ciliates of the three compounds at the highest concentration levels tested. However, the instability of the control microorganism populations prevented valid statistical treatment of the data. A great increase in effort, well above that practical for a project of this nature, would be necessary before strict definitions of toxocity to microorganisms could be determined.

Of the three species of rooted vascular plants tested, only Ludvigia exhibited an interpretable growth pattern. Comparisons between test and control plants indicated no toxicity of any of the compounds tested. No adverse effects on the outward appearance of any of the three species was observed.

The experimental ecosystems were unsuitable for the maintenance of stable macroinvertebrate populations for a duration sufficiently long for compounds testing.

Although considerable information of a qualitative nature was gathered, the flow-through studies did not yield reliable quantitative data from which conclusive determinations of toxicity could be made.

Considering the relative efforts involved, the intermediate-term, flow-through bioassay did not compare favorably to the routine static bioassay as a reliable experimental method.

SECTION XII

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SECTION XIII

REFERENCES

1. Ingols, R.S., and Jacobs, G.M., "BOD Reduction by Chlorination of Phenol and Amino Acids," Sewage and Industrial Wastes, 29, No.3, pp.258-262 (1957).

2. Chambers, C.W., et.al., "Degradation of Aromatic Compounds by Phenol-Adapted Bacteria", Jour. W.P.C.F., 35, No.12, pp.1517-1527 (1963).

3. Ingols, R.S., et.al., "Biological Activity of Halophenols", Jour. W.P.C.F., 38, No.4, pp.629-635 (1966).

4. Hodgman, C.D., et.al., (Editors), Handbook of Chemistry and Physics, 42nd Edition, The Chemical Rubber Publishing Company, Cleveland (1960).

5. Standard Methods for the Examination of Water and Waste-Water, 13th Edition. A.P.H.A., New York, (1971).

6. Roberts, J.D., and Caserio, M.C., Basic Prinicples of Organic Chemistry, W.A. Benjamin Company, New York, (1965).

7. Ettinger, M.B., and Ruchoft, C.C., "Effect of Stepwise Chlorination on Taste- and Odor- Producing Intensity of Some Phenolic Compounds", Jour. A.W.W.A., 43, pp.651ff (1951).

8. Ingols, R.S., and Ridenour, G.M., "The Elimination of Phenolic Tastes by Chloro-Oxidation", Water and Sewage Works, 95, pp.187ff (1949).

9. Burttschell, R.H., et.al., "Chlorine Derivatives of Phenol Causing Taste and Odor", Jour. A.W.W.A., 51, pp. 205-214, (1959).

10. Lee, G.F., "Kinetics of Reactions Between Chlorine and Phenolic Compounds", in Principles and Applications of Water Chemistry, Faust and Hunter (Editors), John Wiley and Sons, New York, (1967).

11. Eisenhauer, H.R., "Oxidation of Phenolic Wastes", Jour. W.P.C.F., 36, No.9, pp.1116-1128 (1964).

12. Weil, I., and Morris, J.C., "Kinetic Studies on the Chloramines. I. The Rates of Formation of Monochloramine, N-Chlormethylamine, and N-Chlorodimethylamine", Journal A.C.S., 71, pp.1664-1671, (1949).

13. Marks, H., by private communication.

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16. Abstract
Fourteen industrial organic chemicals were examined for their persistence through biological treatment, either as the initial compounds, or as degradation products. Semi-continuous activated sludge systems were employed. The ability of each of the chemicals to participate in reactions with free chlorine was then determined in a series of batch experiments.

It was found that certain of the test compounds formed persistent degradation products during treatment. Five of the initial compounds reacted readily with chlorine, under conditions commonly employed in effluent chlorination.

Five of the chlorination products were further studied in respirometer experiments to evaluate their persistence in mixed microbial systems. Their toxicity to fish was determined using the static bioassay procedure.

In the final phase of the study, a series of bench scale, continuous flow ecosystems were established for the evaluation of longer term effects of three of the chlorination products. Several varieties of organisms, representing different levels in the food chain, were studied.

17a. Descriptors

*Chlorination, *Chemical Reactions, *Biodegradation, *Bioassay, *Ecosystems, *Toxicity

17b. Identifiers

*Organic Chemicals, *Activated Sludge, *Degradation Products, *Chlorination Products, *Respirometer Studies, *Continuous Flow Ecosystem

17c. COWRR Field & Group 05B, 05A, 05C

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